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# Influence de la balance hydrique sur les interactions trophiques et le risque de prédation chez un mésoprédateur ectotherme terrestre

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Chloé Chabaud. Influence de la balance hydrique sur les interactions trophiques et le risque de prédation chez un mésoprédateur ectotherme terrestre. Biologie animale. Université de La Rochelle, 2023. Français. NNT : 2023LAROS009 . tel-04368955

**HAL Id: tel-04368955**

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LA ROCHELLE UNIVERSITE

ÉCOLE DOCTORALE

-  
618

Laboratoires : Centre d'études biologiques de Chizé  
& Institut d'écologie et des sciences de l'environnement de Paris

THÈSE

présentée par :

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Soutenue le 21 Avril 2023  
pour l'obtention du grade de Docteur de La Rochelle Université  
Discipline : Biologie de l'environnement, des populations, écologie

**Influence de la balance hydrique sur les interactions trophiques et le risque de  
prédation chez un mésoprédateur ectotherme terrestre**

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*«-Il y a des millions d'années que les fleurs fabriquent des épines. Il y a des millions d'années que les moutons mangent quand même les fleurs. Et ce n'est pas sérieux de chercher à comprendre pourquoi elles se donnent tant de mal pour se fabriquer des épines qui ne servent jamais à rien?*

*Ce n'est pas important la guerre des moutons et des fleurs? Ce n'est pas sérieux et plus important que les additions d'un gros Monsieur rouge? Et si je connais, moi, une fleur unique au monde, qui n'existe nulle part, sauf dans ma planète, et qu'un petit mouton peut anéantir d'un seul coup, comme ça, un matin, sans se rendre compte de ce qu'il fait, ce n'est pas important ça? »*

Le Petit Prince, Antoine de Saint-Exupéry



# Table des matières

## INTRODUCTION

|  |            |
|--|------------|
| I - Changements climatiques et impacts sur les ectothermes.....  | 8          |
| 1. Modifications abiotiques de l'environnement et réponses de la biodiversité.....   | 8          |
| 2. Les ectothermes terrestres en milieu tempéré.....   | 13         |
| 3. Prendre en compte les impacts sur les relations biotiques.....  | 17         |
| II - Balance hydrique chez les ectothermes terrestres.....   | 19         |
| 1. Lien entre balance hydrique et milieux de vie chez les vertébrés ectothermes terrestres   | 19         |
| 2. Lien avec la thermorégulation.....  | 23         |
| 3. Lien avec l'alimentation chez les reptiles terrestres.....  | 24         |
| III- Les comportements anti-prédateurs et leur lien avec l'environnement.....  | 28         |
| 1- Importance des réponses aux prédateurs dans le fonctionnement de l'écosystème.....  | 28         |
| 2- Variation des comportements anti-prédateurs en fonction de facteurs environnementaux.....   | 30         |
| 3- Coûts de ces comportements.....   | 31         |
| Présentation des questionnements de la thèse.....  | 34         |
| <b>Partie I : Variation des traits de thermo-hydrorégulation à l'échelle interspécifique et inter populations.....</b>                                       | <b>48</b>  |
| Chapitre 1) A worldwide and annotated database of evaporative water loss rates in squamate reptiles.....   | 51         |
| Chapitre 2) Climate aridity and habitat drive geographical variation in morphology and thermo-hydroregulation strategies of a widespread lizard species..... | 81         |
| <b>Partie II : Lien entre la balance hydrique et l'alimentation chez un mésoprédateur ectotherme terrestre.....</b>  | <b>135</b> |
| Chapitre 3) Microhabitat humidity rather than food availability drives thermo-hydroregulation responses to drought in a terrestrial lizard.....              | 1367       |
| Chapitre 4) Prey consumption does not restore hydration state but can mitigate the energetic costs of water deprivation in a mesopredator lizard.....        | 173        |

**Partie III : Influence de la balance hydrique sur les comportements anti-prédateurs ..... 203**

    Chapitre 5) Behavioural response to predation risks depends on experimental change in dehydration state in a lizard ..... 205

    Chapitre 6) Hydric and energetic costs of chemoreception: dehydration alters the response to predator odors in a lacertid lizard ..... 229

Discussion et Perspectives..... 250

Remerciements ..... 268

Autres activités réalisées pendant la thèse ..... 274

# INTRODUCTION







## I - Changements climatiques et impacts sur les ectothermes

### **1. Modifications abiotiques de l'environnement et réponses de la biodiversité**

#### **Les effets du changement climatique sur la biodiversité**

Depuis plusieurs décennies, de nombreuses études scientifiques ont démontré que le changement climatique en cours a déjà des effets notables sur la biodiversité et sur le fonctionnement des écosystèmes (Bellard *et al.*, 2012). Les températures moyennes mondiales de surface de l'atmosphère ont augmenté de 1,2 °C depuis l'ère préindustrielle, et les événements météorologiques extrêmes, tels que les inondations, les incendies et les sécheresses, sont aussi plus fréquents et intenses dans de nombreuses régions du monde (IPCC 2021). Ces changements ont eu des conséquences généralisées sur la biodiversité, par exemple sur la répartition spatiale des espèces animales et végétales (Parmesan, 2006; Lenoir *et al.*, 2020), leur phénologie (Parmesan & Yohe, 2003), la dynamique des populations, la structure des communautés et le fonctionnement des écosystèmes (Hooper *et al.*, 2012; Zhou, Wang & Luo, 2020). Malgré des possibles adaptations, environ 25% des espèces de la planète sont actuellement menacées d'extinction en raison du changement climatique à des niveaux divers (IUCN 2022) comme le montrent des études utilisant les projections climatiques futures pour prédire les distributions d'espèces (Thomas *et al.*, 2004). Les espèces sont plus ou moins vulnérables selon leurs traits d'histoire de vie, les plus à risque étant celles qui ont des capacités de dispersion restreintes, les espèces endémiques, les espèces migratrices et les espèces à faible taux de reproduction (Foden *et al.*, 2019). Étudier les effets de ces changements climatiques avec une approche d'écologie évolutive est particulièrement crucial. Les scientifiques prévoient que le changement climatique pèse de plus en plus sur les modifications des écosystèmes et de leurs contributions à l'humanité dans les prochaines décennies, avec des effets variables selon les régions et les scénarios considérés (Thuiller *et al.*, 2005). En effet, les différents scénarios climatiques prévoient majoritairement des effets négatifs des changements climatiques sur la biodiversité, qui s'aggravent, dans certains cas exponentiellement, avec le réchauffement global. Même si celui-ci se limitait à 1,5 ou 2°C, il est susceptible d'entraîner une réduction considérable de la plupart des aires de distribution des espèces terrestres. De plus les effets du réchauffement agissent souvent en synergie avec d'autres menaces telles que la

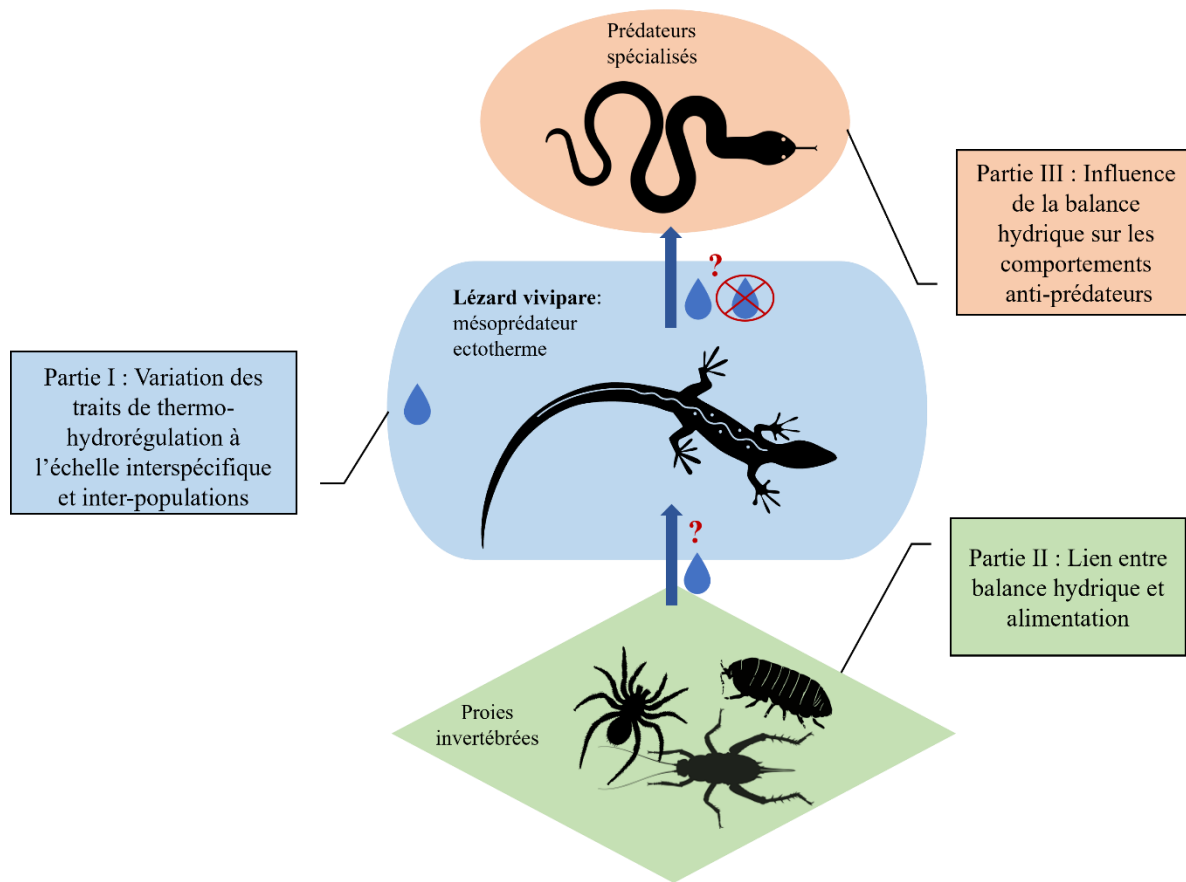
destruction d'habitats, l'introduction d'espèces envahissantes, ou encore la surexploitation des ressources, ce qui contribue à augmenter les risques d'extinction de nombreuses espèces (Stork, 2010; Capdevila *et al.*, 2022).

### **Prendre en compte des facteurs multiples et notamment la disponibilité en eau**

De nombreuses études ont examiné les effets du changement climatique sur les organismes en se concentrant sur les conséquences biologiques et écologiques des modifications de la température de l'environnement. Cependant, les changements climatiques entraînent également une variabilité accrue des précipitations et de la disponibilité en eau des milieux (Dai, 2011; Cook *et al.*, 2014). Ces effets multiples peuvent interagir et avoir des conséquences au-delà de la simple addition des effets des variables (Choi *et al.*, 2019). De plus, les températures et les précipitations varient souvent de façon conjointes (Liu *et al.*, 2009; Hao *et al.*, 2019) et peuvent exercer des contraintes synergiques, en particulier lors de conditions météorologiques extrêmes telles que les canicules et les sécheresses (Ummenhofer & Meehl, 2017).

### **Éléments principaux du projet de thèse**

Dans ce contexte, cette thèse s'intéresse à l'influence de la balance hydrique d'un ectotherme terrestre sur ses interactions trophiques. J'ai choisi de me concentrer sur les effets de la disponibilité en eau dans les milieux naturels comme variable climatique. L'objectif est d'étudier les conséquences qu'une déshydratation chronique peut avoir sur le comportement et la physiologie d'un animal terrestre et comment ces paramètres interagissent avec les relations trophiques. Dans une première partie, nous avons étudié les traits liés à la régulation conjointe de la température corporelle et de la balance hydrique et leurs variations inter et intraspécifique. Dans une seconde partie, nous nous sommes intéressés aux liens entre alimentation et balance hydrique, en se demandant quel rôle jouait la nourriture dans la régulation de la balance hydrique chez un mésoprédateur terrestre. Enfin, dans une troisième partie, nos travaux ont cherché à comprendre les compromis entre balance hydrique et évitement des prédateurs (voir Figure 1). Cette approche est originale car elle associe la recherche en écophysiologie sur les processus de thermo-hydrorégulation à l'échelle d'une espèce et l'écologie des communautés en l'associant à son contexte trophique. Nous avons ainsi tenu compte de son alimentation et de ses prédateurs, deux facteurs qui régulent la dynamique des populations.



**Figure 1 :** Résumé des différents questionnements de la thèse qui cherche à étudier les conséquences de la disponibilité en eau sur un module de la chaîne trophique (lézard mésoprédateur) et sur ses relations avec ses proies ainsi que ses prédateurs. Notre approche écophysiologique se situe à l'échelle d'une espèce mais en l'associant au contexte trophique en tenant compte de son alimentation et de ses prédateurs, qui sont deux facteurs qui régulent la dynamique de populations des lézards.

### **Les impacts du changement climatique concernent différentes échelles de la biodiversité**

Les variations des températures et des précipitations peuvent avoir des conséquences à l'échelle des espèces qui vont migrer vers des zones plus fraîches ou plus humides, mais aussi sur les écosystèmes, entraînant des perturbations dans les relations entre espèces, comme par exemple au sein des réseaux trophiques (Walther, 2010). Ainsi, les changements climatiques affectent tous les niveaux de biodiversité, de la morphologie des individus aux biomes, avec des conséquences sur les individus, les populations, les espèces, les réseaux écologiques et les écosystèmes (Figure 2). Tous ces niveaux sont inter-reliés, et toute perturbation à une échelle entraîne des conséquences

aux niveaux inférieurs et supérieurs (Koh *et al.*, 2004). Par exemple, des changements phénologiques chez les plantes et leurs pollinisateurs peuvent conduire à la co-extinction de plusieurs espèces, ce qui aura des conséquences sur la structure des réseaux plantes-pollinisateurs (Hegland *et al.*, 2009). D'autres modifications de relations interspécifiques peuvent également survenir, telles que la compétition ou les relations proies/prédateurs (Walther, 2010).

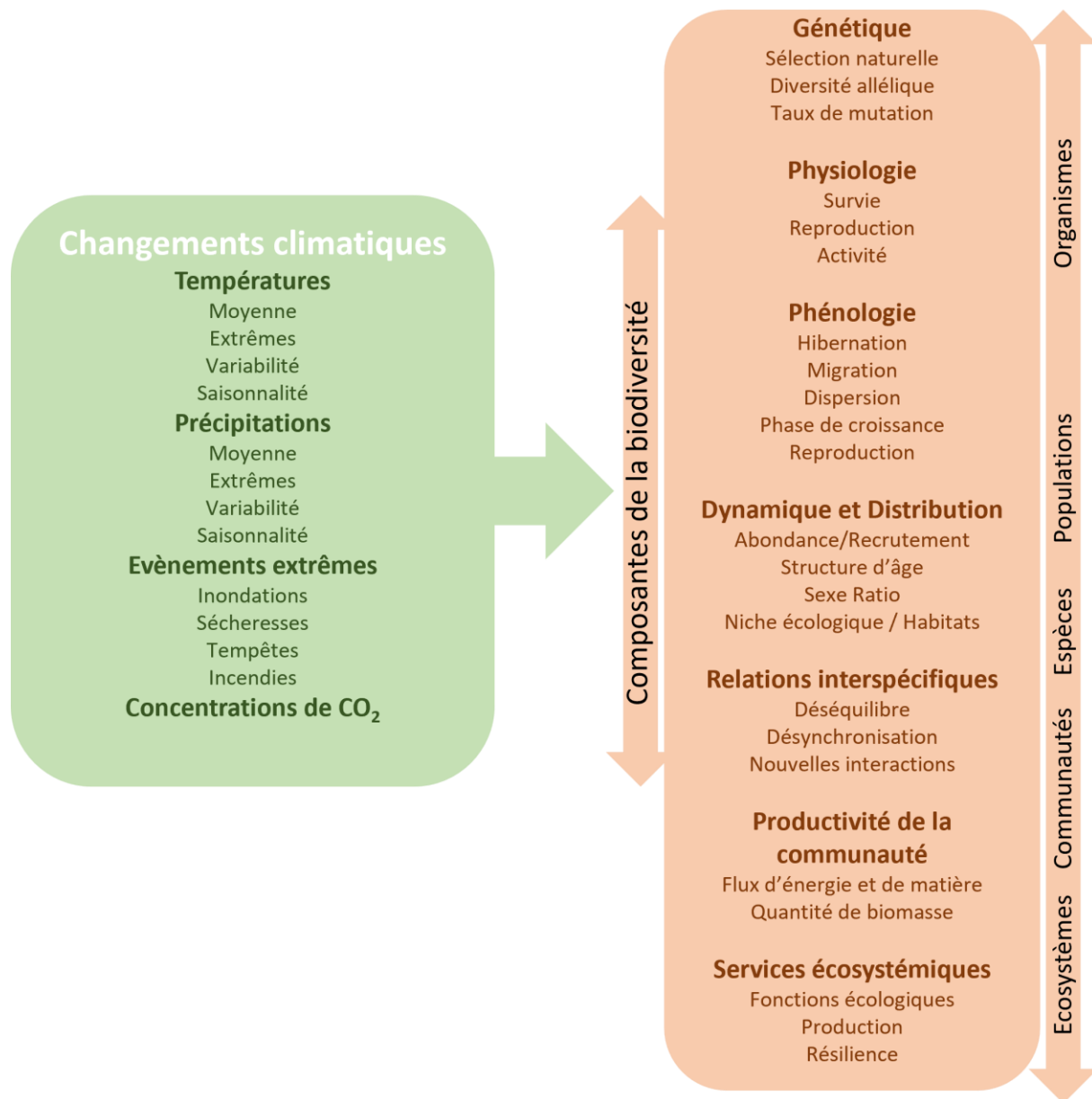
### **Les réponses possibles des espèces à ces changements**

Les espèces « doivent » donc produire des réponses adaptatives pour survivre à ces différents changements de leur environnement abiotique ; ces réponses sont produites par deux principaux mécanismes : la plasticité phénotypique et l'évolution génétique ; et elles peuvent se produire sur trois axes : spatiaux, temporels ou internes (Bellard *et al.*, 2012). Les réponses spatiales impliquent entre autres une migration pour suivre les conditions climatiques optimales pour l'espèce, par exemple en déplaçant l'aire de distribution vers les pôles ou en altitude, tandis que les réponses temporelles impliquent un ajustement de la phénologie pour synchroniser certains événements, comme la date de la saison de reproduction, avec les facteurs climatiques cycliques ou des conditions climatiques rares. Les réponses internes correspondent à des adaptations aux nouvelles conditions climatiques d'ordre physiologique, par exemple pour supporter des environnements plus chauds ou plus secs (Fuller *et al.*, 2010; Seebacher, White & Franklin, 2015; McKechnie & Wolf, 2019), ou d'ordre comportementales comme dans le choix alimentaire ou les patrons d'activité (Silva *et al.*, 2015; Carreira *et al.*, 2016; Mole *et al.*, 2016). Dans le cas d'adaptations génétiques, on s'attend à ce que des pressions de sélection sur la physiologie, la morphologie et le comportement modifient les fréquences génotypiques au sein des populations (Bradshaw & Holzapfel, 2006). Dans le cas de la plasticité phénotypique, les changements environnementaux induisent des modifications flexibles et réversibles de traits morphologiques, physiologiques ou comportementaux, potentiellement adaptatifs, mais qui n'ont pas forcément de base génétique (Charmantier *et al.*, 2008). En particulier, le comportement d'un individu est très plastique et peut changer rapidement en réponse à des modifications de l'environnement (Gross, Pasinelli & Kunc, 2010; Wong & Candolin, 2015). Le comportement peut donc être considéré comme une première interface entre l'environnement et l'organisme. Les individus peuvent ainsi tamponner les effets négatifs des contraintes environnementales pour leur physiologie grâce à des stratégies comportementales (Huey *et al.*, 2012; Gunderson & Stillman, 2015). Etudier les ajustements comportementaux plastiques en réponse aux changements environnementaux est donc

particulièrement important afin de prédire les conséquences de ces modifications sur la biodiversité (Gunderson & Stillman, 2015). Dans cette thèse, je m'intéresse ainsi à approfondir les connaissances sur les comportements participant à la régulation de la balance hydrique individuelle (hydrorégulation) en lien avec les températures corporelles (thermorégulation), dans un contexte de modifications de l'accès à l'eau. En particulier, je cherche à comprendre le lien entre ces traits et les relations trophiques, telles que la prise alimentaire et l'évitement des prédateurs (Figure 1).

### **Comment étudier les réponses des organismes aux changements globaux ?**

Les chercheur•se•s qui étudient comment la biodiversité sera affectée par le changement climatique utilisent le plus souvent des modèles mécanistiques pour faire des projections sur les extinctions ou déplacements d'espèces à différentes échelles (Kearney & Porter, 2009). Or ces modèles ont besoin de paramètres en entrée qui nécessitent des études à l'échelle des populations (comme des suivis démographiques) et à l'échelle individuelle (études physiologiques, comportementales, en conditions contrôlées de laboratoire). Par exemple, il a été montré que la structure et la complexité des microclimats et la capacité des organismes à ajuster leur comportement pour s'y déplacer jouent un rôle déterminant dans la réponse des organismes au changement climatique (Storlie *et al.*, 2014). Il est donc essentiel de déterminer dans quelle mesure les organismes se déplacent et utilisent ces microclimats pour limiter leur exposition aux contraintes climatiques afin de comprendre leurs potentielles réponses et vulnérabilité. Les études de terrain et les expérimentations en laboratoire sont également importantes pour comprendre les effets de changements abiotiques précis et les intégrer dans les modèles. Dans cette optique, l'écophysiologie, à la frontière entre écologie et physiologie des organismes, permet d'étudier les mécanismes proximaux à l'origine des réponses de l'individu aux conditions environnementales et leurs conséquences sur la valeur sélective (survie et reproduction) des individus (Ceia-Hasse *et al.*, 2014). Couplée à l'écologie comportementale, ces deux disciplines apparaissent comme cruciales pour comprendre les raisons sous-jacentes à la distribution des ectothermes terrestres et prédire leurs réponses aux changements climatiques en cours.



**Figure 2 :** Principales composantes abiotiques des changements climatiques en cours et leurs effets sur les composantes des différentes échelles de la biodiversité, adaptée de Bellard *et al.*, 2012

## 2. Les ectothermes terrestres en milieu tempéré

La majorité des espèces animales de la planète sont ectothermes : leur température corporelle dépend des conditions microclimatiques de l'environnement extérieur, elle est donc fluctuante, majoritairement régulée par des comportements de thermorégulation (Angilletta Jr, 2009). En milieu terrestre, les ectothermes incluent les reptiles, les amphibiens et les invertébrés. Ils

présentent des faibles dépenses énergétiques basales (avec un métabolisme au repos 12 à 20 fois plus faible qu'un endotherme de masse équivalente ;Nagy, 2005) et utilisent des moyens essentiellement comportementaux mais aussi secondairement des ajustements physiologiques ou de coloration pour réguler leur température corporelle. Par exemple, ils utilisent la sélection de microclimats leur permettant d'atteindre leur température corporelle préférée ( $T_{pref}$ ) ou encore l'ajustement de leurs postures pour modifier les échanges thermiques avec l'environnement par radiation, conduction ou convection (Angilletta Jr, 2009; Kearney, Shine & Porter, 2009; Woods, Dillon & Pincebourde, 2015). La forte dépendance de leurs stratégies d'histoire de vie et de leur physiologie aux conditions climatiques locales en font des espèces particulièrement sensibles aux changements climatiques (Sinervo *et al.*, 2010; Rohr & Palmer, 2013; Biber, Voskamp & Hof, 2023). La température corporelle joue directement sur leurs performances individuelles à la fois à l'échelle de l'organisme et au niveau cellulaire (Hochachka & Somero, 2002; Kingsolver, Diamond & Buckley, 2013). Les fonctions liées à l'assimilation de ressources ainsi que le métabolisme basal sont particulièrement influencés par les températures extérieures (Schulte, 2015), et susceptibles d'être impactés par des modifications des paramètres climatiques dans le futur (Dillon, Wang & Huey, 2010). Cela aurait des impacts directs sur les performances des individus et sur leurs distributions, si les températures devaient se rapprocher des limites physiologiques que ces espèces sont capables de supporter, appelées communément limites critiques maximales ou  $CT_{max}$  (Huey *et al.*, 2012; Sunday *et al.*, 2014).

Les animaux ectothermes constituent donc de bons modèles pour étudier les impacts des changements environnementaux. Chez ces espèces, l'augmentation des températures environnementales et la modification de la disponibilité en eau constituent deux menaces majeures dues aux changements climatiques dans les écosystèmes terrestres (Sinervo *et al.*, 2010). L'impact des augmentations de températures sur les comportements de thermorégulation et la physiologie a déjà été relativement bien étudié, la thermorégulation comportementale permettant notamment d'éviter des températures extrêmes liées au changement climatique, et le stress thermique associé (Huey & Tewksbury, 2009; Kearney *et al.*, 2009; Woods *et al.*, 2015). De même, les effets du réchauffement sur le taux de croissance des jeunes et leur maturité sexuelle a été étudié précédemment, montrant par exemple que les jeunes lézards grandissent plus rapidement dans un climat plus chaud et accèdent à la reproduction dès l'âge d'un an, au lieu de deux dans les climats plus froids (Bestion *et al.*, 2015). Les travaux récents indiquent que les espèces ectothermes des



zones tempérées sont également très vulnérables au changement climatique en cours (Kubisch, Fernández & Ibarguengoytía, 2016; Biber *et al.*, 2023), bien qu'elles aient été en premier lieu considérées comme moins à risque que les espèces tropicales (Huey *et al.*, 2009; Mi *et al.*, 2022). Etant donnée leur dominance parmi la biodiversité animale terrestre (les ectothermes incluant tous les invertébrés et 80% des vertébrés), il est primordial de comprendre les conséquences des changements globaux sur leur écologie car leur disparition aurait des effets dramatiques dans l'ensemble des écosystèmes terrestres (Cox *et al.*, 2022).

Cependant, nous manquons cruellement de données sur les conséquences écologiques des modifications de la disponibilité en eau et donc de la balance hydrique de ces animaux sur les écosystèmes (McCluney, 2017; Sheridan *et al.*, 2022). Or, il est attendu que les taux de précipitations et la probabilité d'occurrence de sécheresses soient modifiés avec les changements climatiques à venir (Field *et al.*, 2012). Les modifications des régimes de précipitations et les événements extrêmes peuvent aussi affecter fortement les ectothermes terrestres, par exemple en perturbant leur cycle de reproduction, réduisant leur capacité à se nourrir ou encore en modifiant la disponibilité de leurs habitats. En effet les coûts élevés associés à la régulation de la température corporelle et de l'équilibre de la balance hydrique rendent les ectothermes terrestres particulièrement vulnérables, comme l'ont montré des modèles mécanistes récents, mais cela a été encore peu étudié (Pirtle, Tracy & Kearney, 2019; Rozen-Rechels *et al.*, 2019). Les reptiles qui sont majoritairement caractérisés par des petites aires de distribution ou des forts taux d'endémisme comparativement aux autres vertébrés seraient d'autant plus sensibles (Tan, Herrel & Rödder, 2023). C'est pourquoi mes projets de thèse se sont focalisés sur les liens entre balance hydrique et relations trophiques chez un reptile terrestre. Notre modèle d'étude est le lézard vivipare, un mésoprédateur terrestre (i.e. prédateur de taille moyenne et de rang intermédiaire dans le réseau trophique, à l'inverse d'un top prédateur) inféodé aux milieux frais et humides (voir Box 1). Les caractéristiques thermorégulatrices du lézard vivipare, combinées aux connaissances existantes sur cette espèce et à sa capacité d'adaptation aux changements de l'environnement, en font un modèle approprié pour étudier les effets des changements hydrique et thermique de l'environnement sur ses relations trophiques. En effet, ses stratégies de thermorégulation ont été étudiées précédemment, notamment par des travaux de mon équipe à iEES Paris, ce qui permet de bénéficier d'une base de connaissances préalable concernant ses réponses aux changements environnementaux. De plus, cette espèce est considérée comme un thermorégulateur efficace,

capable d'investir beaucoup d'énergie dans la thermorégulation, parfois au détriment des coûts que cela engendre, pour se maintenir dans des milieux peu favorables. Cette espèce peut donc adapter son comportement en fonction des changements de l'environnement, mais cela n'a pas été étudié en tenant compte des relations biotiques qu'il entretient avec les autres espèces de son milieu.



Femelle adulte ©Audrey Ely



Juvenile ©Arnaud Badiane

### **Box 1 : *Zootoca vivipara***

Le lézard vivipare, *Zootoca vivipara* (Lichtenstein, 1823), est une espèce de lézard appartenant à la famille des Lacertidae. Ce petit reptile est bien adapté aux milieux frais et humides, ayant l'une des plus vastes aires de répartition des vertébrés terrestres, allant de l'Europe de l'Ouest jusqu'au Japon, et du cercle polaire jusqu'à la Mer Méditerranée. Le lézard vivipare a une histoire de vie très plastique et est particulièrement sensible aux conditions climatiques, aux pertes hydriques, à la disponibilité de nourriture et à la structure de population. Il entre en hibernation au mois d'octobre, et les mâles adultes sortent les premiers à la fin de l'hiver, suivis par les juvéniles et les femelles.

Les lézards vivipares peuvent en réalité être soit ovipares soit vivipares selon les populations. Chez les vivipares, les femelles produisent généralement une portée par an d'en moyenne 5 à 6 œufs non-calciifiés. Les juvéniles viables sortent de l'œuf quelques minutes à quelques heures après la ponte et sont immédiatement indépendants. Leur croissance est continue, mais a lieu en majorité pendant les deux premières années de la vie. Les mâles et les femelles peuvent accéder à la reproduction dès l'âge d'un an dans les climats les plus chauds, et ont des capacités de dispersion limitées, de l'ordre de quelques dizaines de mètres.

Le lézard vivipare est un mésoprédateur opportuniste qui consomme une large gamme d'arthropodes incluant des araignées, des insectes, des vers et des isopodes terrestres. Il est prédaté par des oiseaux et des serpents qui vivent dans son environnement, comme par exemple les vipères péliades ou les coronelles lisses.

### 3. Prendre en compte les impacts sur les relations biotiques

Les effets du changement climatique ne se limitent pas à une seule espèce, mais peuvent avoir des conséquences en cascade sur l'ensemble d'un écosystème (Walther, 2010), bien que les études omettent souvent de s'intéresser aux impacts du climat sur ces interactions biotiques. Pourtant, les interactions entre les espèces sont cruciales pour leur survie, et les changements environnementaux en cours peuvent entraîner des modifications de ces interactions (Valiente-Banuet *et al.*, 2015), qu'elles soient mutualistes (symbiotiques par exemple) ou antagonistes (relation proie-prédateur par exemple, voir résumé dans Tylianakis *et al.*, 2008). Par exemple, l'avance de la phénologie des insectes en réponse à l'augmentation des températures affecte la synchronisation entre la reproduction des oiseaux insectivores et l'accès à leur nourriture (Durant *et al.*, 2007). Des effets au niveau microbien ont aussi été observés, comme en témoigne la forte baisse de la diversité bactérienne chez les lézards en réponse à une augmentation de la température (Bestion *et al.*, 2017). Parmi les interactions interspécifiques, les relations proies-prédateurs jouent un rôle majeur dans la dynamique des populations et communautés et dans l'évolution de traits d'histoire de vie (e.g., Doligez & Clobert, 2003). Les changements climatiques peuvent impacter ces relations de différentes manières : (1) en modifiant les abondances d'espèces de proies, ce qui peut ensuite avoir des conséquences en cascade sur tout le réseau (Bretagnolle & Terraube, 2019) ; (2) en changeant spatialement ou dans le temps la distribution des proies, provoquant une inadéquation avec les besoins des prédateurs (Logan, Wolesensky & Joern, 2006; Durant *et al.*, 2007); (3) en modifiant les comportements et la physiologie des prédateurs ou des proies. Ce dernier point peut se traduire par des changements de patrons d'activité en réponse à une augmentation des températures, altérant les probabilités de rencontre entre proies et prédateurs (Menge & Olson, 1990) ou induire un besoin énergétique plus important chez les prédateurs ectothermes alors même que l'abondance des proies est réduite (Vasseur & McCann, 2005). Les prédateurs de sommet de chaîne sont d'autant plus vulnérables aux changements climatiques à cause de ces effets sur tous les échelons inférieurs du réseau trophique dont ils dépendent, dont les extinctions dites secondaires (Voigt *et al.*, 2003). De plus, les réseaux trophiques sont sensibles aux changements de phénologie ou d'aire de distribution d'espèces (Both *et al.*, 2009). Une approche holistique qui intègre les différents niveaux d'organisation de la biodiversité est donc essentielle pour comprendre les effets du changement climatique sur les écosystèmes (Gilman *et al.*, 2010; Montoya & Raffaelli, 2010). Dans le cadre de

cette thèse, je me suis intéressée aux relations trophiques, en choisissant un modèle d'étude mésoprédateur, qui me permet d'étudier à la fois sa sensibilité en tant que prédateur (lien entre l'alimentation et la balance hydrique et la thermorégulation, Partie 2) et en tant que proie (influence de la balance hydrique sur l'évitement des prédateurs, Partie 3). En effet, les lézards jouent un rôle central dans les réseaux trophiques des écosystèmes tempérés où ils sont à la fois prédateurs de multiples espèces d'invertébrés en général (Meiri, 2018) et eux-mêmes la proie de plusieurs prédateurs (Carretero, 2004), parmi lesquels on trouve en Europe les rapaces, les corvidés, les grands mammifères, les serpents et les chats domestiques en milieu urbain (e.g., Prestt, 1971; Woods, McDonald & Harris, 2003; Steen, Løw & Sonerud, 2011; Drobenkov, 2014; Pérez-Mellado *et al.*, 2014). De plus, les lézards peuvent développer des stratégies d'évitement des prédateurs pour faire face aux différents types de prédation (autotomie de la queue, chémoreception, voir par exemple Van Damme *et al.*, 1995; Bateman & Fleming, 2009). Ce sont donc de bons modèles pour étudier les effets biotiques sur les relations proies-prédateurs. De plus, *Zootoca vivipara* se nourrit principalement d'insectes, qui sont en fort déclin dû aux changements climatiques (Harvey *et al.*, 2022). Dans le futur, la contrainte hydrique à laquelle les lézards devront faire face sera donc très certainement couplée à une plus faible densité de proies. Etudier les effets conjoints d'une restriction hydrique et alimentaire sur leurs comportements, leur physiologie et leur reproduction présente donc un intérêt notoire (voir **Chapitre 3** et Discussion).

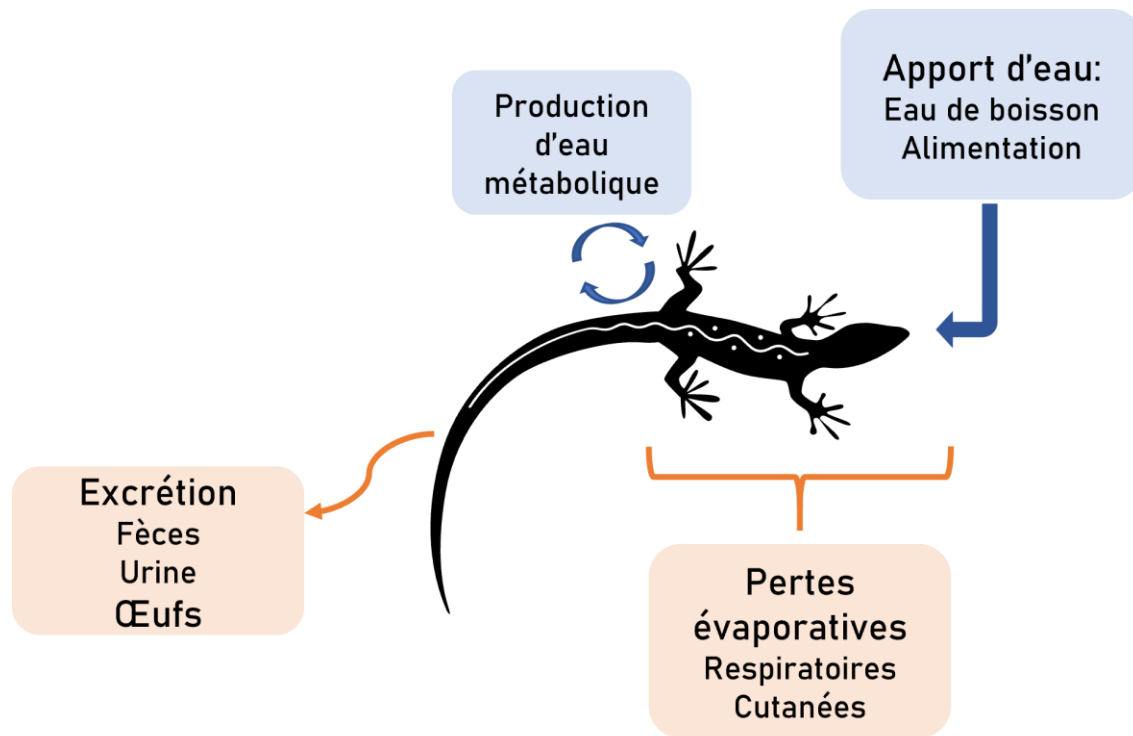
L'effet de l'eau sur les interactions écologiques a été peu étudié, mais quelques études soulignent l'importance de la balance hydrique des animaux pour les réseaux trophiques (Spiller & Schoener, 2008; McCluney & Sabo, 2016). Dans une étude en mésocosmes, McCluney & Sabo (2016) ont notamment montré une modification de l'effet top-down des prédateurs sur l'abondance d'herbivores et la végétation, lorsque ceux-ci n'avaient pas accès à de l'eau libre, suggérant que la balance hydrique des prédateurs est un facteur important de la dynamique des interactions trophiques dans les écosystèmes. Une autre étude suggère même que l'eau pourrait être le moteur principal des interactions trophiques dans les milieux terrestres où l'eau libre n'est pas permanente, plutôt que l'énergie ou les nutriments (McCluney & Sabo, 2009). Bien que l'eau puisse aussi avoir un effet bottom-up via son impact sur la végétation, Deguines, Brashares & Prugh (2017) ont montré dans un écosystème de prairie semi-aride que les effets indirects (via la faune) des précipitations sur les interactions biotiques prévalaient sur les effets médiés par la végétation. Il

semble donc urgent d'améliorer nos connaissances des effets de l'eau et de la balance hydrique sur les relations biotiques dans les écosystèmes terrestres (McCluney, 2017).

## II - Balance hydrique chez les ectothermes terrestres

### **1. Lien entre balance hydrique et milieux de vie chez les vertébrés ectothermes terrestres**

La régulation de la balance hydrique chez les ectothermes terrestres résulte d'un équilibre subtil et précaire entre les apports d'eau et les pertes en eau (Figure 3). L'apport d'eau dépend de plusieurs sources en fonction des espèces et des milieux de vie (Shoemaker & Nagy, 1977), notamment l'eau libre ou eau de boisson (Karasov & del Rio, 2007), de l'eau contenue dans les aliments (Nagy *et al.*, 1991) et de l'eau métabolique (Jindra & Sehnal, 1990). Les pertes d'eau se produisent principalement par l'excrétion, l'évaporation à travers la peau, lors de la respiration et via les produits de reproduction (Shoemaker & Nagy, 1977). Les contraintes imposées par le bilan hydrique influencent l'évolution de traits physiologiques et comportementaux de la même manière que le font les températures, affectant par exemple le temps d'activité et le choix d'habitat des reptiles (Pintor, Schwarzkopf & Krockenberger, 2016; Pirtle *et al.*, 2019). On parle donc de traits d'hydrorégulation pour décrire les mécanismes permettant de réguler les flux hydriques entre l'animal et son environnement (Rozen-Rechels *et al.*, 2019). Ces traits peuvent varier en fonction de l'environnement, notamment les pertes hydriques évaporatives (cutanées et respiratoires) sont très variables selon les espèces et parfois même au sein des espèces en fonction des conditions environnementales (Buttemer & Thomas, 2003). Afin de conserver une balance hydrique optimale, les ectothermes terrestres vont pouvoir jouer sur trois mécanismes principaux : (1) maintenir un apport d'eau optimal via l'eau de boisson (Shoemaker & Nagy, 1977) ou éventuellement l'alimentation (Znari & Nagy, 1997) ; (2) limiter les pertes hydriques grâce à des changements de patrons d'activité, de choix d'habitats ou de structure de la peau (Agugliaro & Reinert, 2005; Lillywhite, 2006; Davis & DeNardo, 2010; Chown, Sørensen & Terblanche, 2011; Wegener, Gartner & Losos, 2014; Dezetter, Le Galliard & Lourdaï, 2022) ; (3) produire de l'eau métabolique (Nagy, 1972), en passant par exemple par la mobilisation de l'eau liée aux protéines contenues dans les muscles (Brusch *et al.*, 2018) ou par le métabolisme respiratoire.



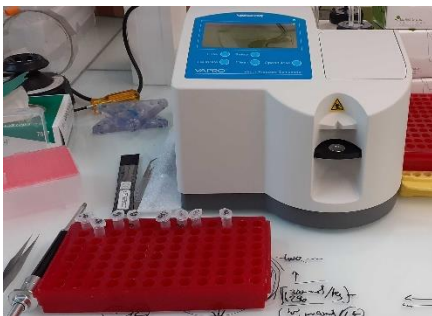
**Figure 3** : Composantes de la balance hydrique d'un ectotherme terrestre, avec les voies d'acquisition d'eau en bleu et les pertes d'eau en orange.

Ainsi, de la même manière que pour la thermorégulation, les performances de l'organisme sont maximales lorsque l'hydrorégulation est optimisée, mais elles diminuent lorsque l'organisme se déshydrate (Figure 4). Bien que moins étudiée que les effets de la température, la disponibilité en eau peut notamment contraindre fortement les performances locomotrices ou la reproduction de certaines espèces de reptiles via ses effets sur la balance hydrique et le statut d'hydratation de l'individu (Nagy *et al.*, 1991; Peterson, 1996; Dupoué *et al.*, 2018). Cependant, contrairement à la thermorégulation, peu de travaux portent sur les mécanismes de l'hydrorégulation et la manière dont ceux-ci sont liés aux autres contraintes que rencontrent les ectothermes (thermorégulation, accès aux ressources, évitement des prédateurs). Pour l'étudier, il faut prendre en compte l'état hydrique de l'animal, qui peut se mesurer chez les reptiles grâce à la combinaison de l'osmolalité plasmatique et la masse corporelle (voir Box 2). Pour le faire varier, nous avons au cours de cette thèse manipulé deux paramètres lors des études expérimentales : l'accès à l'eau de boisson (accès ou non à une coupelle d'eau dans le terrarium ou l'arène expérimentale) et l'humidité de

l'environnement (par le nombre de vaporisation par jour du milieu expérimental ou par l'humidité relative dans les abris). En effet, dans la nature, l'accès à l'eau libre dépend de la présence ou non dans l'environnement d'un point d'eau permanent, ou en l'absence d'un tel point, de l'apparition de flaques ou points d'eau temporaires liés aux précipitations. Dans ce dernier cas, la présence d'eau libre dans l'environnement peut dépendre de la saison, des événements extrêmes tels que les sécheresses estivales, ou encore de l'action de l'humain sur les habitats (assèchement de mare, drainage des zones humides). Le maintien de la balance hydrique repose aussi sur l'humidité de l'air dans l'environnement ou dans les abris à disposition, pour limiter les pertes hydriques liées au gradient de vapeur d'eau entre le milieu intérieur de l'animal et l'air autour de lui. Dans l'étude de terrain présentée dans le **Chapitre 2**, nous avons choisi de prendre en compte ces deux aspects, en sélectionnant des populations avec ou sans accès à l'eau permanent, couplé à des populations d'aridité variable (calculée avec l'indice d'Emberger sur les mois d'activité du lézard), et en essayant de les décorrélérer. De plus, la base de données présentée dans le **Chapitre 1** nous permettra d'investiguer plus précisément les liens entre traits de la balance hydrique (pertes évaporatives totales, cutanées) et climat, en prenant en compte les biais liés à la phylogénie.

### Box 2 : Méthodes de mesure de paramètres liés à la balance hydrique

Osmolalité plasmatique comme indicateur de la balance hydrique individuelle (mOsm.kg<sup>-1</sup>)



Osmomètre à tension de vapeur

L'osmolalité plasmatique mesure la quantité de solutés, tels que les ions et les particules libres, présents dans le plasma sanguin par kilogramme. Elle reflète donc la concentration de solutés dans le sang. Elle augmente lorsque le volume d'eau dans le sang diminue, ce qui peut se produire en cas de déshydratation. Ainsi, une osmolalité élevée indique un état de déshydratation, plus précisément lorsqu'elle est associée à une perte de masse corporelle. Pour mesurer l'osmolalité plasmatique, j'ai utilisé un osmomètre à tension de vapeur qui fonctionne en mesurant la tension de vapeur de l'eau dans l'échantillon de plasma, qui est directement liée à la concentration de solutés. J'ai utilisé des échantillons de 6 µL de plasma de lézard, dilués dans du sérum physiologique pour obtenir 2 répliques de 10 µL chacun. Des expériences précédentes ont montré que la valeur basale chez cette espèce est d'environ 320 mOsm.kg<sup>-1</sup> et qu'une déshydratation modérée augmente l'osmolalité de 20 à 50 mOsm.kg<sup>-1</sup> et une forte déshydratation de 100 mOsm.kg<sup>-1</sup>.



## Pertes hydriques cutanées



Exemple de mesure des pertes hydriques cutanées et contention du lézard vivipare adulte

On mesure le flux hydrique évaporatif transcutané surfacique avec une chambre d'accumulation (Aquaflux BIOX). Cet instrument consiste en une chambre de mesure close sans mouvement d'air équipée d'un condenseur (une surface de plafond maintenue à  $-7.5^{\circ}\text{C}$  environ) qui convertit de manière continue l'eau évaporée en glace et à mi-hauteur d'un capteur de température et d'humidité relative. La mesure du gradient d'eau entre la surface de la peau (considérée comme partiellement humide) et la surface du condenseur (considérée comme sèche) permet de calculer le flux d'eau à l'aide de la première loi de diffusion de Fick. Un embout adapté à la petite taille du lézard (Nail Cap) a été utilisé pour adapter l'appareil au lézard vivipare. Le calibrage se fait avec un bouchon d'étalonnage fermé qui ne laisse pas passer d'air afin que le flux se stabilise vers la valeur 0. Après le calibrage, on met l'embout Nail Cap et on tient l'animal par ses deux pattes droites, de façon à ce qu'il soit contre notre pouce gauche. On place ensuite la sonde fermement contre la peau dorsale de l'animal de façon à faire un joint étanche mais sans altérer le flux sanguin. On commence la lecture du flux. La courbe de réponse doit ressembler à une cloche qui diminue de façon logarithmique. On lit la valeur lorsque 10 lectures successives ont une différence inférieure à 0.01%.

## Pertes hydriques évaporatives totales (couplées au métabolisme au repos)



Dispositif de mesure du métabolisme et des pertes hydriques totales au repos en flux fermé

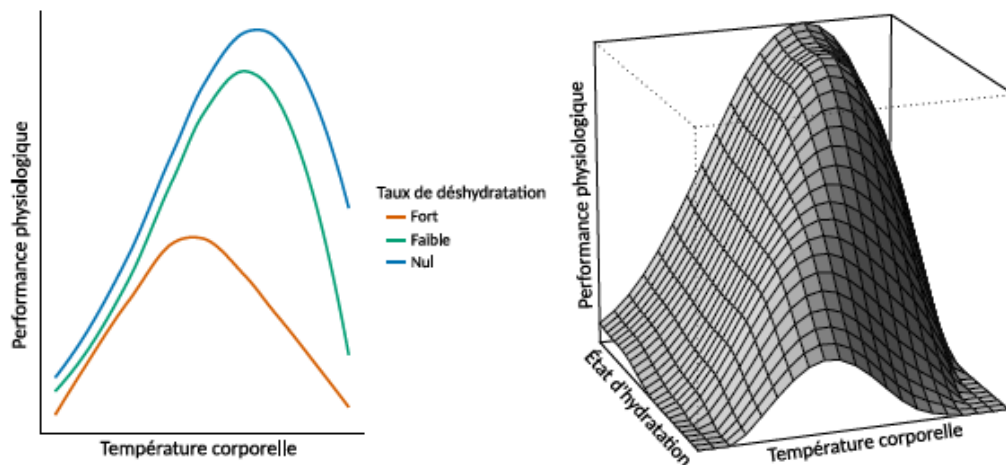
Une des façons de mesurer les pertes évaporatives totales est lors de mesures du métabolisme basal sur des lézards maintenus à jeun (pour éviter l'influence des excréments). J'ai mesuré celui-ci par calorimétrie indirecte et conjointement à une mesure de perte hydrique par bilan de masse en enceinte climatique à température contrôlée. Pour cela, les lézards sont pesés avec une balance de haute précision (0,01mg, Sartorius). Ils sont ensuite placés dans des bocaux en verre de 1L, connectés à un distributeur d'air venant d'une pompe prélevant l'air d'une pièce annexe non occupée. Les bocaux sont placés dans une chambre climatique maintenue à  $20^{\circ}\text{C}$ . Les animaux sont laissés deux heures en acclimatation avec le bocal entrouvert et l'air distribué. Puis nous fermons les bocaux et faisons un flush de 5 minutes pour s'assurer de renouveler entièrement l'air du bocal. Après le flush, les valves sont fermées et nous laissons les individus environ 12 heures au repos dans les bocaux. Ensuite, nous prélevons deux seringues de 140mL d'air par bocal après avoir homogénéisé l'air du bocal par deux allers-retours de pompage. Nous pesons ensuite de nouveau les animaux en notant la présence éventuelle de fèces. On analyse la concentration volumétrique d' $\text{O}_2$  et de  $\text{CO}_2$  dans chaque paire de seringues de chaque bocal et dans des seringues de référence avant et après la série. Les données de concentration sont utilisées pour calculer le volume d'oxygène et de  $\text{CO}_2$  échangé par heure et quotient respirométrique (QR). La



|  |   |
|--|---|
|  | différence de masse nous donne les pertes hydriques au repos en absence de fèces. |
|--|---|

## 2. Lien avec la thermorégulation

La régulation de la balance hydrique est intimement liée à la régulation de la température corporelle chez les ectothermes. En effet, les pertes d'eau par évaporation augmentent avec la température corporelle, le métabolisme, la respiration et les conditions thermiques de l'environnement (Woods & Smith, 2010). La régulation conjointe de ces deux paramètres est décrite par le concept de thermo-hydrorégulation, dont les implications éco-évolutives ont été étudiées dans Rozen-Rechels *et al.* (2019). Les stratégies mises en place pour réguler ces deux paramètres conjointement impliquent des comportements variés et des compromis entre les deux (Pintor *et al.*, 2016). Par exemple, le choix d'un habitat doit permettre de prendre en compte à la fois les risques de déshydratation et les besoins métaboliques de l'animal (Riddell *et al.*, 2018; Rozen-Rechels *et al.*, 2020). La déshydratation peut notamment compromettre le maintien d'une température optimale et donc des performances de l'individu (Anderson & Andrade, 2017, Figure 4). Etudier l'effet de variables environnementales sur les traits liés à la thermo-hydrorégulation implique donc de comparer des environnements qui sont contrastés à la fois sur le plan thermique et sur le plan hydrique, en essayant de décorrélérer ces deux paramètres qui varient souvent de façon conjointe (Kelley *et al.*, 2015). C'est ce que nous avons essayé de faire en comparant des populations de lézards vivipares dans un gradient climatique allant de la marge sud en plaine à des populations de hautes altitudes (**Chapitre 2**). Pour cela nous avons choisi des milieux contrastés à la fois au niveau d'un indice d'aridité global prenant en compte les précipitations moyennes sur la période d'activité de cette espèce, mais aussi sur les températures.



**Figure 4 :** Courbes de performance théoriques d’un ectotherme terrestre, en fonction des variations conjointes de la balance hydrique et de la température, mettant en évidence les compromis de la thermo-hydrorégulation, issue de Rozen-Rechels, 2019.

### 3. Lien avec l’alimentation chez les reptiles terrestres

L’acquisition de nourriture et son utilisation pour la croissance et le maintien des paramètres physiologiques est l’une des tâches les plus importantes dans la vie d’un organisme (Anderson, 2007). Le régime alimentaire d’une espèce dépend de nombreux paramètres comme la morphologie du système digestif (Iverson, 1982), de traits physiologiques (Beaupre, Dunham & Overall, 1993), ou encore de variables environnementales ou saisonnières (Vitt, 1991). A l’échelle individuelle, dans la Partie 2, j’ai investigué les compromis entre l’alimentation et la balance hydrique chez les reptiles terrestres. En effet, des études ont montré que certains reptiles terrestres s’adaptent à leur environnement en utilisant des stratégies alimentaires ou physiologiques qui leur permettent de compenser une absence de ressource en eau libre, et qui peuvent varier en fonction de l’habitat et du climat (Gienger, Tracy & Nagy, 2014). C’est particulièrement le cas des reptiles des régions arides ou extrêmes qui ont tendance à avoir des régimes alimentaires plus spécialistes, tandis que les reptiles des zones tropicales et tempérées ont tendance à avoir des régimes alimentaires plus généralistes (Ocampo *et al.*, 2022). La majorité des lézards sont carnivores à base d’invertébrés (Table 1, Meiri, 2018), aussi appelés insectivores, ce qui rend notre modèle d’étude, le lézard vivipare, intéressant pour étudier les liens entre balance hydrique et alimentation. En effet, il se

nourrit d'une grande diversité d'invertébrés et son régime alimentaire est flexible et peut être modifié par des augmentations de températures, notamment vers une plus grande proportion de proies prédateurs et une réduction des proies phytophages (Vacheva & Naumov, 2019; Bestion *et al.*, 2019).

| Régime alimentaire | Régime plus précis        | Pourcentage d'espèces |
|--------------------|---------------------------|-----------------------|
| Carnivore          | Dont des vertébrés        | 4%                    |
| Carnivore          | Seulement des invertébrés | 76%                   |
| Omnivore           | Animaux et végétaux       | 15%                   |
| Herbivore          | Végétaux                  | 5%                    |

**Table 1 :** Régime alimentaire des espèces de lézards, adaptée de Meiri, 2018

Cependant peu d'études ont considéré le rôle de l'alimentation dans la régulation de la balance hydrique individuelle, et souvent de manière indirecte et chez des espèces de milieux secs (voir Table 1 du **Chapitre 4**). En résumé, il semblerait que les régimes alimentaires à base de plantes ou d'insectes permettraient aux reptiles d'obtenir de l'eau via leur nourriture, mais ce n'est pas le cas pour les carnivores stricts (la majorité des serpents et certains lézards). Au contraire, pour les espèces carnivores des milieux secs étudiées, manger augmente le comportement de boisson. Toutefois, aucune étude ne concerne les insectivores ou omnivores habitant des milieux tempérés, n'ayant donc pas *a priori* d'adaptation à la sécheresse. Si le réchauffement augmente la dépendance des ectothermes à la nutrition (Huey & Kingsolver, 2019), nous faisons l'hypothèse que le manque d'eau peut avoir un effet similaire, alors même que la diminution des précipitations affecte les insectes dont dépend l'alimentation des insectivores (Sánchez-Bayo & Wyckhuys, 2019). Nous avons réalisé deux études complémentaires pour étudier à la fois la composante comportementale et les mécanismes physiologiques qui relient la balance hydrique et la prise de nourriture. Dans le **Chapitre 4**, nous avons réalisé une étude expérimentale afin de déterminer le rôle de l'alimentation dans le maintien de la balance hydrique physiologique, et les potentiels compromis entre balance énergétique et hydrique chez un ectotherme terrestre. Afin de suivre l'état de santé et de mobilisation des ressources chez les lézards, nous mesurons en plus des variations de masse

corporelle plusieurs autres variables morphologiques, qui sont de bons indicateurs de la mobilisation de réserves pour compenser la déshydratation ou le jeun (voir Box 3).

Si la nourriture permet un apport d'eau pour ces animaux des milieux tempérés, un shift alimentaire est une réponse possible au changement de disponibilité en eau dans les milieux, ce qui a des implications pour la dynamique des écosystèmes (Bestion *et al.*, 2019). Des traits liés au régime alimentaire (comme le degré de généralisme ou la capacité à modifier son régime alimentaire) pourraient faire partie des possibles adaptations aux changements globaux, rendant les espèces plus ou moins vulnérables (Durtsche, 2000). Une étude a par exemple démontré que les espèces qui changeaient leur distribution vers les hautes altitudes étaient plus généralistes comparées aux espèces déjà présentes en altitude (Lurgi, López & Montoya, 2012).

Les compromis entre balance hydrique et énergétique peuvent aussi se situer au niveau des comportements. De la même manière qu'il existe un trade-off entre la thermorégulation et l'hydrorégulation, la recherche de nourriture, la prise alimentaire et les mécanismes de digestion qui nécessitent une température élevée peuvent entrer en conflit avec l'hydrorégulation lorsque l'eau vient à manquer. Nous avons testé ce compromis dans le **Chapitre 3** avec une étude expérimentale combinant une restriction en eau et en nourriture et en observant les changements de comportements et d'utilisation d'abri ainsi provoqués.

Enfin, nous avons étudié les effets conjoints d'une restriction alimentaire et hydrique lors de la gestation sur les performances de reproduction. Cette étude permet de comprendre comment ces conflits peuvent interagir lors de cette étape essentielle du cycle de vie des organismes qui a un impact direct sur leur valeur sélective et sur la dynamique des populations (voir Discussion 4.).

Box 3 : Mesures morphologiques de suivi de mobilisation de ressources chez le lézard vivipare



Largeur de queue, proxy des réserves caudales

La mesure de l'épaisseur de la queue au niveau de la 8<sup>ème</sup> rangée d'écaillés caudales au cours d'une manipulation permet de suivre la mobilisation des réserves de lipides et de muscles stockés dans la queue. Ils peuvent permettre de compenser une restriction hydrique ou nutritive en libérant des triglycérides ou de l'eau liée aux protéines. Pour faire cette mesure, nous scannons les animaux en vue ventrale puis nous analysons les images avec le logiciel ImageJ. Nous choisissons une rangée assez éloignée du cloaque afin de ne pas mesurer les hemipénis chez les mâles, dont la taille varie au cours de la saison.



Epaisseur des muscles des pattes arrière, proxy de la mobilisation de protéines musculaires

Nous mesurons l'épaisseur des muscles des membres postérieurs pour évaluer une éventuellement mobilisation des muscles lors de restrictions hydrique et/ou alimentaire. En effet, les réserves de lipides sont privilégiées lors d'un jeun pour fournir de l'énergie, mais les muscles peuvent notamment aider à maintenir la balance hydrique, car ils sont plus efficaces que les graisses à libérer de l'eau liée. Pour cela nous utilisons un spessimètre digital que nous plaçons en dessous et au-dessus de la patte du lézard.

### III- Les comportements anti-prédateurs et leur lien avec l'environnement

#### **1- Importance des réponses aux prédateurs dans le fonctionnement de l'écosystème**

Les prédateurs ont des effets directs (mortalité) et indirects (effets non-consommateurs) sur leurs proies, notamment sur leur taille de population, leurs comportements et l'utilisation de l'espace. Au-delà des effets directs historiquement très étudiés sur la densité de population de proies (Berryman, 1992), les prédateurs induisent par leur présence un stress, amenant les proies à développer des stratégies d'évitement ou de défense, appelées comportements anti-prédateurs. Cette présence des prédateurs induit des changements de comportements, de physiologie et parfois de morphologie qui jouent un rôle au moins aussi important sur la fitness des proies que l'effet direct de la prédation (Peckarsky *et al.*, 2008; Sheriff *et al.*, 2020). Par exemple, une expérience chez des têtards a montré un compromis dans la variation du comportement en réponse à un risque de prédation couplé à une disponibilité de ressources limitée. Intégrer ces réponses simultanées à différentes pressions permettrait de mieux modéliser la dynamique de population (Anholt & Werner, 1995). Un autre exemple issu des expériences de cascade trophique dans des lacs du Wisconsin a montré que la diminution de l'espèce de proie dominante venait au moins autant de son changement de comportement suite à l'introduction de prédateurs que de l'effet consommateur des prédateurs (He & Kitchell, 1990; Peckarsky *et al.*, 2008). Le stress physiologique causé par la pression de prédation peut aussi jouer sur la reproduction des espèces proies (Forsgren, 1992), et donc modifier significativement leur densité de population, là encore en dehors des effets directs de consommation (Boonstra *et al.*, 1998). La présence de prédateurs peut également affecter les comportements de recherche de nourriture (Beckerman, Wieski & Baird, 2007), ou les mécanismes physiologiques responsables de l'efficacité de conversion (McPeck, 2004) et donc modifier la croissance et le développement des proies. Plus largement, ces effets peuvent avoir des implications sur la dynamique des nutriments dans l'écosystème, en modifiant les besoins énergétiques des herbivores (Hawlena & Schmitz, 2010).

Il est intéressant d'étudier comment les proies intègrent le risque de prédation dans leurs décisions comportementales (Lima & Dill, 1990; Lima, 1998), et de révéler les potentiels compromis qui existent entre l'évitement des prédateurs et la nécessité de remplir les besoins fondamentaux tels

que se nourrir, thermoréguler, se reproduire. On parle d'écologie de la peur pour décrire les réponses des proies, et les coûts non-léthaux de la présence de prédateurs sur leurs comportements, alimentation, physiologie et sur les communautés (Brown, Laundre & Gurung, 1999; Clinchy, Sheriff & Zanette, 2013; Gaynor *et al.*, 2019). Dans un environnement contrasté, le risque de prédation varie dans l'espace, et les proies vont construire ce qu'on appelle un paysage de la peur en fonction de leur perception du risque de prédation. La perception du risque dépend de la capacité des proies à détecter les prédateurs, de leur connaissance du danger ou encore des indices laissés par les prédateurs. Le comportement des proies va ensuite intégrer ces informations, couplées à l'évaluation des coûts et des bénéfices d'une réponse comportementale, pour prendre une décision sur son utilisation de l'espace, son activité et éventuellement la mise en place d'un comportement anti-prédateur (Lima & Dill, 1990; Gaynor *et al.*, 2019). Les lézards utilisent à la fois la vue et la chémoréception pour détecter leurs prédateurs et les éviter efficacement (Amo, López & Martín, 2004b). Ils expriment ensuite une variété de comportements anti-prédateurs, dont ceux bien documentés pour le lézard vivipare sont résumés dans la Box 4.

**Box 4 : Comportements anti-prédateurs de *Zootoca vivipara***

Présentation des variables comportementales mesurées dans la littérature pour évaluer la réponse de *Zootoca vivipara* ou d'autres espèces proches de Lacertidae à un prédateur spécialiste

|                           |  |
|---------------------------|--|
| <b>Temps de latence</b>   | Nombre de secondes entre la présentation d'une odeur et la première réaction   |
| <b>Tongue Flick</b>       | Coup de langue à l'extérieur du museau qui permet l'échantillonnage de molécules chimiques dans l'environnement proche |
| <b>Fuite</b>              | Mouvement rapide en vue de s'éloigner de la source d'odeur   |
| <b>Vibration de queue</b> | Mouvement rapide de la queue pour attirer l'attention du prédateur sur cette partie du corps                           |
| <b>Secousse de patte</b>  | Patte antérieure tapée contre le sol de façon répétée  |
| <b>Morsure</b>            | Attaque et morsure envers l'objet servant à la présentation de l'odeur   |

|                       |  |
|-----------------------|--|
| <b>Immobilisation</b> | L'animal est figé et se maintient immobile                                 |
| <b>Grattage</b>       | Gratte avec ses pattes antérieures les bords de l'arène comme pour creuser |
| <b>Sursaut</b>        | Réaction soudaine souvent suivie d'une courte course                       |

**Issus des études suivantes :** Thoen, Bauwens & Verheyen, 1986; Van Damme *et al.*, 1995; Amo, López & Martín, 2004

## 2- Variation des comportements anti-prédateurs en fonction de facteurs environnementaux

Ces comportements anti-prédateurs sont dépendants du contexte environnemental et individuel. Leur apparition et leur fréquence peuvent être influencées premièrement par des facteurs environnementaux. C'est le cas par exemple des températures lors du développement (Culler, McPeck & Ayres, 2014; Seebacher & Grigalchik, 2015), des ressources disponibles dans le milieu (Houston, McNamara & Hutchinson, 1997), de la structure de la végétation (Woodley & Peterson, 2003), de l'exposition à un contaminant (ex : le cadmium, Sornom *et al.*, 2012; le zinc, Janssens *et al.*, 2014) ou encore de l'hypoxie qui altère la fuite ou la formation de bancs qui est un comportement de défense contre les prédateurs chez les poissons (Domenici, Lefrançois & Shingles, 2007). Comprendre comment les changements climatiques vont impacter ces relations entre proies et prédateurs nécessite d'intégrer plusieurs facteurs (Domenici *et al.*, 2019). En effet, pour les animaux vivant dans des milieux aux températures plus basses que leur optimum, l'augmentation des températures peut donc avoir un effet bénéfique sur leur survie en leur permettant de mieux répondre aux prédateurs (Matassa & Trussell, 2015). Au contraire, chez d'autres espèces le changement climatique et l'augmentation des températures pourraient accroître les effets négatifs des prédateurs sur la chaîne trophique, dits top-down, et toucher plus fortement les consommateurs intermédiaires (Miller, Matassa & Trussell, 2014). Deuxièmement, des facteurs inhérents à la proie peuvent modifier ces comportements, par exemple l'âge de l'individu si la réponse au prédateur provient d'un apprentissage, son rôle social pour les espèces ayant des individus dédiés à la surveillance et à l'alerte, ou encore l'état de santé (Wirsing *et al.*, 2021).

La majorité des études portant sur les facteurs impactant les comportements anti-prédateurs sont réalisées sur des espèces de milieux aquatique et marin, et peu sur des écosystèmes terrestres. De



ce fait, très peu de travaux se sont intéressés au rôle de l'eau dans la variation de ces réponses aux prédateurs, avec une majorité de travaux portant sur des endothermes dans des milieux arides et semi-arides, en particulier les communautés d'ongulés et de carnivores (Valeix *et al.*, 2008; Molokwu *et al.*, 2010). Chez les ongulés, l'eau intervient par exemple par la complémentarité dans l'habitat de la distribution de l'eau et des prédateurs, lorsque les points d'eau sont également des lieux à fort risque de prédation (Valeix *et al.*, 2009). Mes travaux de thèse visent donc à comprendre si la variation de la disponibilité de l'eau dans le milieu peut influencer les comportements anti-prédateurs ou si l'état hydrique de l'animal peut modifier sa réponse aux prédateurs. Pour cela, j'ai réalisé une expérience combinant un stress hydrique et un risque de prédation et observé les modifications de comportements de thermo-hydrorégulation que cela engendre chez le lézard vivipare (**Chapitre 5**).

Chez le lézard vivipare, des travaux précédents ont montré que la chémoréception et notamment le nombre de *tongue flicks* (voir Box 4) était influencée par la température corporelle (Van Damme *et al.*, 1990). Nous avons voulu savoir si l'état hydrique de l'animal pouvait aussi influencer ce comportement, en comparant le nombre de *tongue flicks* en réponse à une odeur de prédateur chez des lézards déshydratés et bien hydratés (**Chapitre 6**).

### **3- Coûts de ces comportements**

Ces réponses face aux prédateurs ont des coûts non négligeables pour les proies, qui impactent leur valeur sélective. Ceux-ci peuvent être de différentes natures : des coûts énergétiques que ce soit pour synthétiser des substances chimiques de défense ou d'alarme (Smith, 1992) ou produire des cris ou des comportements défensifs qui consomment de l'énergie comme la fuite (LaGory, 1987) ; des coûts temporels car l'évitement des prédateurs peut faire perdre à la proie des opportunités de thermoréguler, d'obtenir de la nourriture ou d'autres ressources ; mais aussi des coûts sociaux (Downes & Shine, 1998) et des impacts sur les défenses immunitaires (Rigby & Jokela, 2000). Enfin, de nombreuses études ont mis en évidence les impacts de ces stratégies anti-prédateurs sur la croissance et la reproduction (Nelson, 2007), qui peuvent passer par des opportunités ratées

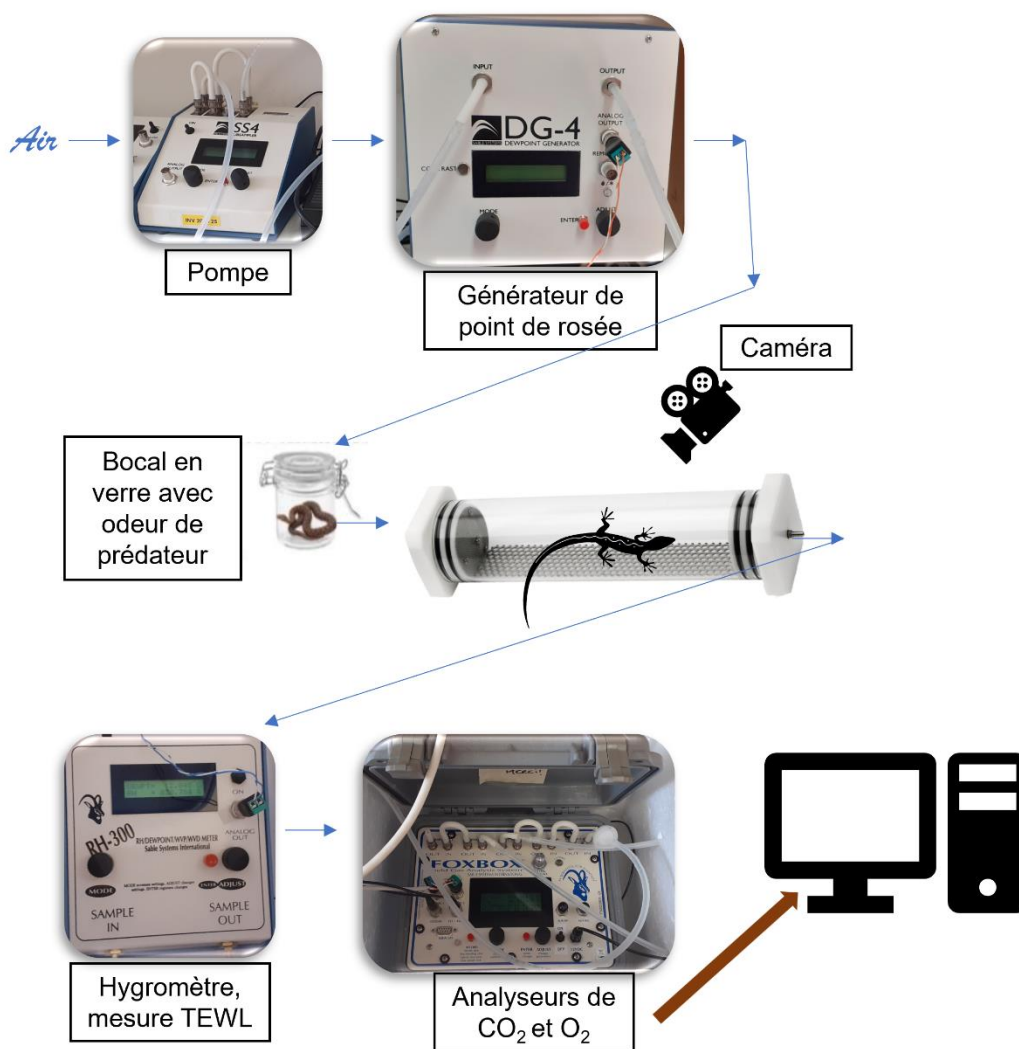
d'accouplement, ou des effets du stress sur les descendants (Martin, 2011; MacLeod *et al.*, 2022). Les compromis entre thermorégulation et évitement des prédateurs chez les ectothermes ont été étudiés précédemment, montrant que souvent les comportements permettant d'éviter les prédateurs sont prioritaires sur les besoins de thermoréguler (Downes, 2001; Gvoždík, Černická & Van Damme, 2013).

Chez les reptiles, la détection de prédateurs est permise par la vue mais aussi par la chémoréception, un système sensoriel impliquant l'organe voméronasal et qui permet à ces espèces de détecter des substances chimiques volatiles dans l'environnement (Evans, 2003). C'est d'ailleurs pour échantillonner les substances chimiques que les lézards sortent leur langue fourchue et réalisent des *tongue flicks*. Or les coûts associés à ce comportement n'ont pas été étudiés. Chez des reptiles sensibles aux pertes hydriques évaporatives, comme c'est le cas de notre modèle d'étude le lézard vivipare (Dupoué *et al.*, 2017), on pourrait s'attendre à ce que ce comportement provoque des pertes hydriques non négligeables (liées à l'ouverture de la bouche et à l'extension de la langue, comme c'est le cas pour le halètement, voir Loughran & Wolf, 2023). Nous avons donc cherché à évaluer les coûts associés à la détection de prédateur et à l'augmentation du taux de *tongue flicks* chez le lézard vivipare, grâce à des mesures métaboliques et de pertes hydriques dans le **Chapitre 6** (voir Box 5).

Dans le **Chapitre 5**, nous avons étudié comment les comportements sont modifiés lorsque les contraintes hydriques et le risque de prédation sont conjoints. Le coût hydrique des comportements de prédation peuvent générer des effets de priorité où le risque de prédation serait dominant sur le risque de déshydratation dans la décision de comportement à adopter et donc annulerait les réponses comportementales liées à la déshydratation, comme cela a pu être montré par rapport à la régulation de la température (Downes, 2001). Mais cela peut aussi impacter les comportements anti-prédateurs par des effets de condition-dépendance, si l'état de déshydratation est un déterminant de ces réponses anti-prédateurs.

### Box 5 : Respirométrie en flux ouvert pour mesurer les coûts énergétique et hydrique des réponses à une odeur de prédateur

Nous avons utilisé le système de respirométrie présenté dans la Box 2 en flux ouvert pour calculer les coûts énergétique et hydrique d'une augmentation du taux de *tongue flicks*. L'air extérieur est amené par une pompe dans un générateur d'humidité dont on règle le point de rosée, qui permet de contrôler l'humidité de l'air entrant pour pouvoir ensuite mesurer la différence induite par le passage de l'air au contact du lézard. Puis l'air traverse un bocal saturé en odeurs de prédateurs grâce à des mues de serpents. Ensuite l'air entre dans la chambre de mesure dans laquelle le lézard se trouve. L'air sortant est analysé par un hygromètre qui permet de déterminer l'eau libérée par le lézard puis par des analyseurs de gaz qui donnent les concentrations en oxygène et CO<sub>2</sub>.



## Présentation des questionnements de la thèse

Dans la partie I, nous avons cherché à comprendre comment les traits morphologiques et physiologiques liés à la balance hydrique chez les reptiles squamates varient en fonction de facteurs environnementaux. Dans le **Chapitre 1**, nous avons travaillé sur une base de données rassemblant de nombreuses études ayant mesuré les pertes en eau par évaporation chez 34 familles de squamates, répartis sur une grande partie du globe, ainsi que des données issues de travaux non publiés de nos collaborateurs. Nous présentons les enjeux de ces données pour mieux comprendre les variations géographiques de ces traits, en prenant en compte des facteurs phylogénétiques, mais aussi les potentiels biais venus des méthodes de mesure ou des conditions expérimentales. Le **Chapitre 2** présente une étude comparative à l'échelle de populations d'une même espèce, *Zootoca vivipara*, mais vivant dans des environnements climatiques et des habitats contrastés. Nous avons échantillonné 15 populations allant de la marge chaude au sud de la distribution de cette espèce à des populations plus en altitude, et mesuré des traits liés à la thermo-hydrorégulation pour comprendre quel facteur environnemental influençait prioritairement ces traits. En particulier, nous avons cherché à décorrélérer température et accès à l'eau afin de tester l'influence de ces deux paramètres séparément.

L'objectif de ma thèse était ensuite d'intégrer les effets de la balance hydrique sur les relations interspécifiques, je suis donc passée à des expériences à l'échelle individuelle pour comprendre les mécanismes physiologiques et comportementaux liant balance hydrique et alimentation et balance hydrique et risque de prédation. Dans la partie II, nous avons d'abord étudié les compromis comportementaux permettant de réguler à la fois la balance énergétique et la balance hydrique en observant l'influence de la qualité hydrique de l'habitat sur les comportements d'un lézard terrestre en réponse à une sécheresse et/ou une restriction alimentaire. Les résultats sont présentés dans le **Chapitre 3**, et ont été produits lors du stage de master 2 de Théo Bodineau. Dans le **Chapitre 4**, j'ai réalisé une expérience pour évaluer le rôle de la nourriture dans le maintien de la balance hydrique chez un lézard insectivore de milieu tempéré. J'ai suivi l'état de déshydratation de lézards en comparant des groupes ayant accès à de la nourriture avec des groupes à jeun pour comprendre s'ils pouvaient extraire de l'eau de leurs proies.

La partie III nous a permis d'étudier l'influence de la balance hydrique sur l'autre aspect des relations proies-prédateurs du lézard vivipare, c'est-à-dire en tant que proie, par ses capacités à détecter et éviter ses prédateurs. Dans le **Chapitre 5**, j'ai relevé les comportements de lézards confrontés conjointement à un stress hydrique et à un risque de prédation. L'objectif était d'étudier s'il existe des interactions entre ces deux contraintes sur les comportements de thermorégulation, et si l'hydrorégulation comportementale était affectée par la présence de prédateurs dans le milieu. Dans le **Chapitre 6**, j'ai cherché à mesurer les coûts physiologiques d'un comportement anti-prédateur bien connu chez les squamates, le *tongue flick*, et évaluer comment l'état hydrique de l'animal pouvait affecter sa capacité à répondre à la présence de prédateur.

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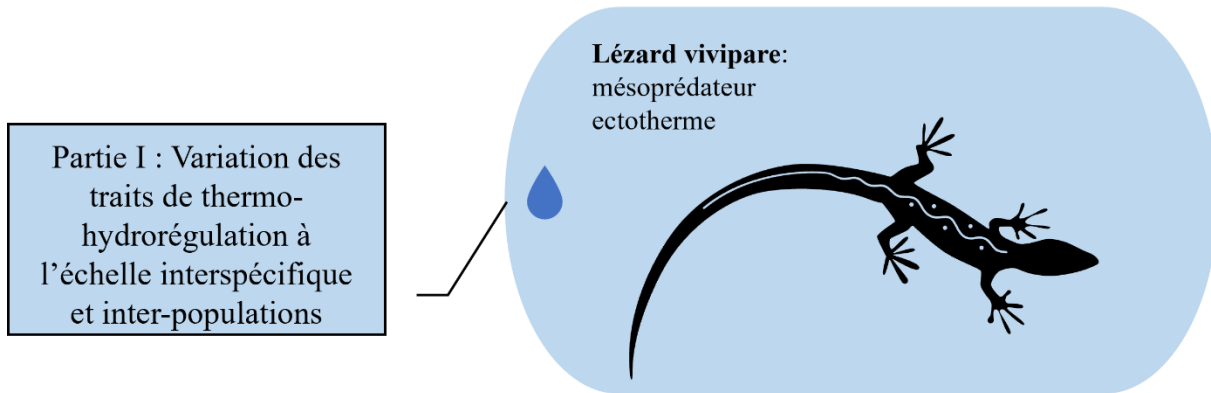
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Partie I : Variation des traits de thermo-hydrorégulation à l'échelle interspécifique et inter populations



## Résumé :

Cette première partie comprend deux chapitres déjà publiés qui décrivent la variation de traits écophysiologiques liés à la régulation de la balance hydrique chez les reptiles squamates.

Le premier est un data paper qui présente une base de données « SquamEWL » sur les pertes en eau évaporatives à l'échelle interspécifique, rassemblant des données sur plus de 300 espèces de reptiles squamates répartis sur tous les continents, dans des milieux très variables en terme d'aridité. Des données complémentaires, dont les taux métaboliques, sont fournies pour de futures recherches. L'article présente des statistiques descriptives préliminaires, identifie les lacunes et les pistes prometteuses pour enrichir et explorer cette base de données. Son utilisation permettra des recherches futures sur les adaptations fonctionnelles des traits liés à la balance hydrique en rapport avec des paramètres individuels (taille, masse corporelle) et environnementaux (aridité).

Le deuxième chapitre s'intéresse à la variation de ces traits à l'échelle intraspécifique chez le lézard vivipare (*Zootoca vivipara*), en comparant 15 populations réparties sur un fort gradient climatique dans le sud de la France. Les conditions thermiques et hydriques locales sont des facteurs importants de la variation géographique des stratégies de thermorégulation et d'hydrorégulation, mais également les différents types d'habitat, qui sont aussi recensés dans cette étude à travers l'accès à l'eau libre et la couverture forestière. Des mâles adultes ont été échantillonnés pour des traits morphologiques, leur taux métabolique au repos, les pertes en eau évaporatives totales et les pertes en eau cutanées, ainsi que les préférences thermiques. Mis à part une diminution de la taille corporelle des adultes avec l'augmentation des températures environnementales, peu d'effets des conditions thermiques sur les stratégies de thermo-hydrorégulation ont été détectés. En particulier, les populations reliques des régions basses en altitude et plus chaudes ne présentent pas d'adaptations écophysiologiques spécifiques. En revanche, la condition corporelle et le taux métabolique au repos sont positivement associés à un gradient de précipitations, tandis que la couverture forestière et l'accès à l'eau dans l'habitat tout au long de la saison influencent particulièrement les pertes hydriques totales. Cette étude souligne l'importance des précipitations et des caractéristiques de l'habitat plutôt que des conditions thermiques dans la variation géographique de la morphologie et de la physiologie des lézards liée à la thermo-hydrorégulation.



## **Chapitre 1) A worldwide and annotated database of evaporative water loss rates in squamate reptiles**

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*Published in Global Ecology and Biogeography in 2021*

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## **Abstract**

**Motivation:** The understanding of physiological adaptations along environmental gradients, of evolutionary radiation in past and present environments and of recent ecological responses to global change calls for global, comprehensive databases of the functional traits of extant organisms. The ability to maintain an adequate water balance is a critical functional property determining the resilience of animal species to climate variation. In terrestrial or semi-terrestrial organisms, total water loss (TWL) is largely dominated by evaporative water loss (EWL). The analysis of geographic and phylogenetic patterns of variation in EWL rates must however account for differences in methods and potential confounding factors known to influence standard measures of whole-organism water loss. Here, we compiled the global and standardized SquamEWL database of total, respiratory and cutaneous EWL for 302 species of Squamate reptiles (895 samples and 2533 estimates) from across the globe. We also present preliminary descriptive statistics for the compiled data, discuss methodological caveats and identify promising avenues to update, expand and explore this database.

**Main types of variables contained:** standard water loss rates, geographic and phylogenetic data.

**Spatial location:** global.

**Time period:** data were obtained from extant species and were collected between 1932 and 2020.

**Major taxa:** Reptilia, Squamata including lizards, snakes and amphisbaenians.

**Level of measurements:** individual samples of animals from the same species, locality, size class and sex category.

**Software format:** csv.

**Keywords:** ectotherms, functional traits, homeostasis, hydroregulation, macrophysiology, evaporative water loss.

## 1. Background and summary

The ecological niche of a species reflects its position within an ecosystem and depends on abiotic, biotic and historical conditions (Chase & Leibold, 2003). The interactions between organisms and their abiotic environment determine the fundamental niche. Their mechanistic characterization and dynamics have become fundamental topics in global change biology (Araújo & Guisan, 2006; Sinervo *et al.*, 2010; Boyle *et al.*, 2020). As the fundamental niche depends in part on organismal traits, current research aims to determine the life history characteristics, physiological limits and behavioral traits constraining species distributions (Kearney & Porter, 2009; Kearney *et al.*, 2010). The compilation of databases of functional traits, defined as the morphological, physiological, phenological or behavioral traits that determine the performance of individuals (Violle *et al.*, 2007), is a fundamental step in this research program (Schneider *et al.*, 2019). Furthermore, global databases of functional traits can be used to investigate universal scaling rules and advance our understanding of evolutionary processes (Díaz *et al.*, 2016; Etard *et al.*, 2020). Research on the climatic niches of ectothermic animals over the last decades has focused on the study of functional traits characterizing the thermal biology, including thermal limits (Sunday *et al.*, 2012; Bennett *et al.*, 2018), thermal performance curves and metabolism (Dillon *et al.*, 2010), thermoregulation behavior (Kearney *et al.*, 2009), or thermal sensitivity of development (Noble *et al.*, 2018). However, studies have shown that traits associated to water balance in ectotherms are also critical in setting their climatic niche, their sensitivity to global changes, and their macro-evolutionary radiation patterns (Brischoux *et al.*, 2012; Garcia-Porta *et al.*, 2019; Gouveia *et al.*, 2019; Rozen-Rechels *et al.*, 2019; Lertzman-Lepofsky *et al.*, 2020).

In animals, water balance is maintained by a combination of morphological features and physiological and behavioral mechanisms (e.g., behavioral hydroregulation, skin resistance to water loss or respiration, Chown *et al.*, 2011; Pintor *et al.*, 2016; Pirtle *et al.*, 2019; Riddell *et al.*, 2019) that equate water gain versus water losses. As a result, body hydration state is regulated within a safety zone to avoid the acute and chronic, potentially lethal, effects of dehydration (Figure 1A). In this regard, an intrinsic vulnerability common to all terrestrial and semi-terrestrial organisms is that body water is at a permanent risk of being lost by evaporation. Total evaporative loss (TEWL) comprises both the water lost through the skin epidermis or exoskeleton (cutaneous water loss, CWL) and during gas exchanges via the respiratory system (respiratory water loss, RWL), with the partitioning between them varying considerably among different animal groups.

Although functional traits such as desiccation resistance and behavior are also important for hydroregulation, EWL rate has proven to be a relevant metric to address water regulation strategies, and the susceptibility of organisms to drought, habitat aridity or salinity, in birds (Albright *et al.*, 2017; Boyle *et al.*, 2020), mammals (Van Sant *et al.*, 2012), non-avian reptiles (Brischoux *et al.*, 2012; Cox & Cox, 2015), amphibians (Lertzman-Lepofsky *et al.*, 2020) and insects (Addo-Bediako *et al.*, 2001). However, to our knowledge, there has been no attempt to compile and annotate a global database of all published TEWL records in terrestrial animals, including those of Squamate reptiles.

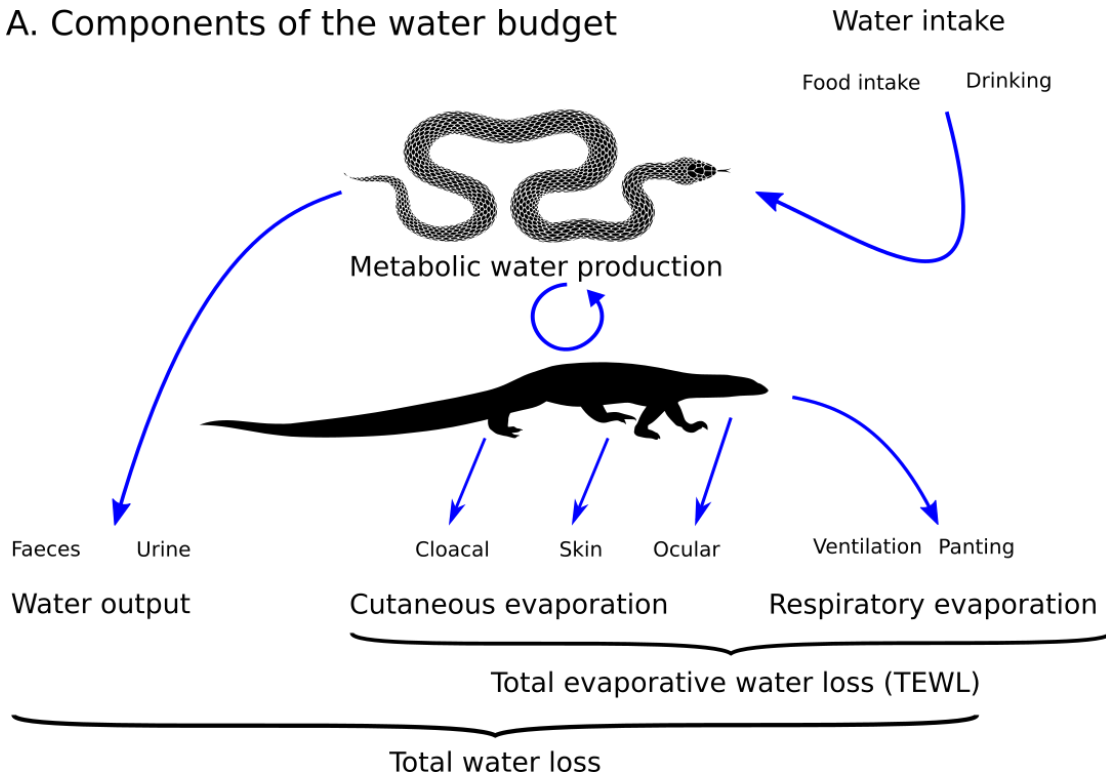
Squamate reptiles are ideal model systems to improve our understanding of water regulation strategies since these dry-skinned ectothermic organisms share proximate mechanisms of water loss (Mautz, 1982), and exhibit great taxonomical and ecological diversification, broad variation in body size and shape, and are found in most habitats across the globe (Meiri, 2018). In addition, their performance and life history strategies are greatly influenced by the water budget, which is primarily determined by EWL and water inputs from food and free standing water (Kearney & Porter, 2004; Lillywhite, 2017; Rozen-Rechels *et al.*, 2020). In Squamates, the rates of TEWL (Mautz, 1980) vary with micro-climatic conditions, morphological and functional adaptations, life stages and behavioral strategies (e.g., space use and activity, Pirtle *et al.*, 2019). EWL is not as strongly involved in thermoregulation as in endothermic animals: heat loss due to CWL and RWL is generally negligible in Squamate reptiles, except under extreme conditions such as panting in some desert species or under extreme heat stress (Tattersall *et al.*, 2006; Loughran & Wolf, 2020). Although CWL is generally the dominant avenue of water loss in Squamate reptiles, the partitioning between RWL and CWL, including trans-epidermal, ocular and cloacal water loss, varies between and within species (Mautz, 1982; Pirtle *et al.*, 2019). Changes in patterns and rates of EWL can rapidly evolve in Squamates, and there is evidence of adaptive plastic responses of TEWL to fluctuating temperatures or hydric conditions (Kattan & Lillywhite, 1989; Moen *et al.*, 2005; Cox & Cox, 2015; Garcia-Porta *et al.*, 2019; Sannolo *et al.*, 2020). Yet, estimates of TEWL are influenced by methodological choices, sampling methods (e.g., size class or seasonal factors), acclimation conditions and reporting methods, which makes comparisons across studies difficult without an unambiguously defined vocabulary and broad set of standardized metadata (see Figure 1B).



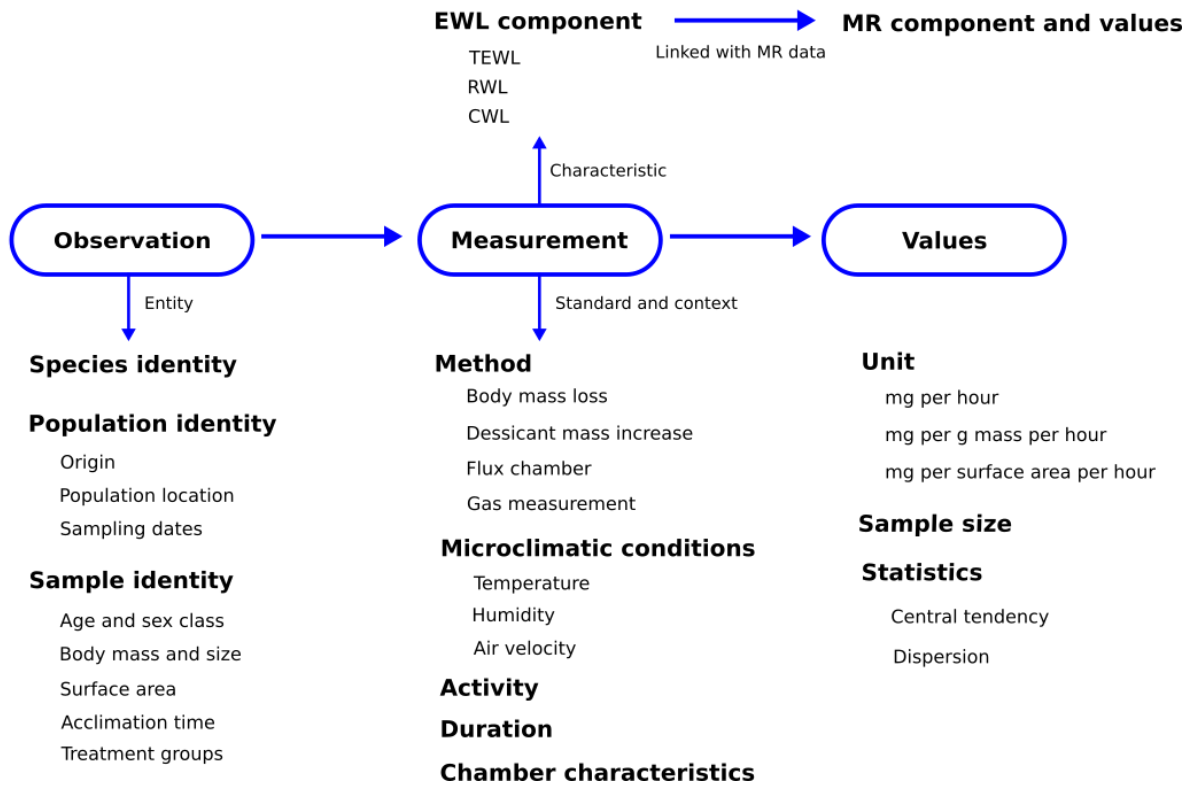
Here, we assembled a global and annotated database of standard rates of evaporative water loss in Squamate reptiles of the world using published information and unpublished data collected by us in recent years. Our initiative differs from previously published data sets by (1) its wide taxonomic scope spanning all available data for all Squamate reptiles including lizards, snakes and amphisbaenians, (2) its exhaustiveness since we gathered all identified published estimates from each method and recorded samples and units from the original publications without *a priori* exclusion of particular data or *ad hoc* calculations of statistics, and (3) its large comprehensiveness since we compiled information on 28 metadata variables and provide a computing script to facilitate future use of these data. We hope that making this data freely available will stimulate future research on water balance in reptiles, particularly on water conservation mechanisms and even more so on the geographic, ecological, and phylogenetic correlates of evaporative water loss.

**Figure 1.** Components of water loss in Squamate reptiles and metadata required to describe evaporative water loss (EWL). **A.** The total water budget of the animal rests on balance between water intake from food and drinking of rain, free standing water or moisture, metabolic water production and water loss from respiratory evaporation (RWL), water loss from skin, ocular or cloacal evaporation (CWL) and water loss from feces and excreta. Total evaporative water loss (TEWL) is the sum of total respiratory and cutaneous evaporative water loss and can be measured from post-absorptive animals in controlled laboratory conditions provided they do not produce feces or excreta. **B.** Controlled description of EWL rates data using metadata describing properties of entities (observations, including species, population and animals), observations (including characteristics, protocols and context) and values. A rich metadata set is especially needed to describe the methodology and the environmental conditions when EWL was measured. Each concept in this figure refers to one or several columns of metadata in our database (see Table 1 for details).

## A. Components of the water budget



## B. Controlled description of EWL



## 2. Methods and dataset

We searched for published literature and referenced reports providing potential data on water loss rates in Squamate reptiles in Web of Science and Google Scholar using relevant search terms in title, abstract and content with the following query: (“water loss” OR “water balance” OR “hydroregulation”) AND (“reptile\*” OR “snake\*” OR “lizard\*” OR “squamate\*”). In addition, we extracted all references from a recent comparative analysis of water loss in reptiles (Cox & Cox, 2015). The availability of water loss data on samples of individuals (excluding eggs or embryos) from known reptile species was then checked by a single person (JFLG) who stored all such references, source files as well as available online data in a Zotero group library (see <https://tinyurl.com/y2nclru5>). Using tags, all publications were then assigned to a single person who oversaw confirming availability of water loss data, extracting the data and adding relevant metadata to a spreadsheet. If additional relevant publications were identified, those were added to the Zotero library and processed by the same individual. This procedure was performed first in September 2018 and repeated in October 2019, February 2020, and September 2020 and spans data sources published from 1932 to late 2020. Additionally, unpublished data were also made contributed by all authors.

We produced a library of 159 publications, reports or academic contributions (monographs, dissertations and thesis) from which we extracted complete or partial data (see Supplementary File 1). EWL data was then added into a spreadsheet together with all the available metadata describing the relevant conditions of water loss measurements, species and sample characteristics, and contextual information regarding animal morphology, location, sampling dates and habitat. The species and subspecies identity was standardized using the EMBL/EBI Reptile DataBase release of December 21, 2019 (Uetz & Etzold, 1996). We performed data extraction accepting a sample data point as defined by a unique group of animals composed of a fixed set of individuals, subjected to the same experimental protocol, and measured under the same conditions. For each sample, we extracted the mean and dispersion statistics (SD, SE or range) of EWL rates (total water loss, respiratory water loss or cutaneous water loss) and the mean and dispersion statistics (SD, SE, or range) of body mass as well as mean statistics for body size (snout to vent and total length) and body surface area. Data were taken from published or shared datasets, extracted from tables and text, or extracted from figures using scanned images of the plots and the Plot Digitizer program in Java (<https://sourceforge.net/projects/plotdigitizer/>). Information on measurement method,

measurement temperature, water vapor pressure deficit (VPD), air flow, measurement duration, and activity statuses of animals were extracted whenever available or requested from the corresponding authors. All data is presented as values on the scales chosen for reporting, although, whenever possible, the possibility for scale conversion is provided (see below).

We also included variables describing study design (groups and treatments) and a quality score permitting the exclusion of data (abnormal animals, animals maintained under manipulated conditions or measurements performed with non-standard conditions) in future extraction and analysis. Each of us scored a study data quality ranging from high (appropriate protocols, protocols are well reported, and data statistics are detailed), medium (one item is missing) to low (poorly designed and reported protocols and poorly detailed data statistics). The same person also scored the data standards as either usable to not usable data (because of low study standards or inappropriate metadata, see detailed comments available in a free text item). The content of the publicly available database is outlined in Table 1, which provides additional information on each field of the data table. Upon data extraction, each record was checked by the data collectors and the content and integrity of the whole database was checked by two individuals prior to uploading the first version, called SquamEWL, in a public repository (doi: 10.5281/zenodo.3666172). Routines for data extraction, database integrity check and data cleaning were coded in the R statistical language, and are briefly described below. The fully annotated code written for R version 3.6.3 (R Core Team, 2020) is also available in the public repository and includes functions to convert records between measurement scales.

### **3. Preliminary analyses**

The SquamEWL dataset includes 2533 water loss records in 347 species and sub-species of Squamate reptiles in 895 unique samples (mean number of individuals per sample =  $10.15 \pm 11.5$  SD, median = 6, range=1-169) with most measurements obtained for TEWL (N = 2170) and substantially fewer for CWL (237) and RWL (126). The vast majority of records is from field-captured animals (N = 2013) in comparison to laboratory acclimated animals or those raised in outdoor enclosures (202). There is substantial variation in the acclimation time of animals (time spent in the laboratory prior to measurement), even after excluding laboratory-raised animals (range=0-750 days, mean =  $46.45 \pm 119.4$  SD, median = 7). The predominant protocol involves measurements of body mass loss in laboratory (N = 1474), followed by direct measurements of

water vapor changes in flow-through chambers (674), measurements of the mass increase of a desiccant (296), doubly-labeled water techniques in active animals (56) and, in more recent studies, flux chamber protocols for measurements of trans-epidermal water loss (33). The variation in micro-climatic environmental conditions during sampling is wide with air temperatures ranging from 5.3 to 45°C (mean =  $28 \pm 6.47$  SD, median = 27), VPD ranging from ca. 0 to 9.10 kPa vapor pressure deficit (mean =  $2.83 \pm 1.73$  SD, median = 2.53) and air flow ranging from 0 to several hundred mL per min (mean =  $120.7 \pm 244.4$  SD, about half of the records were obtained in still air).

The geographic origin is available for 1921 records in 314 unique localities (Figure 2A) and those records are predominantly located in Northern and Central America, Europe and Australia, with under-representation of samples for pan-tropical diversity hotspots for those species in South America, Africa and Asia as well as several semi-arid and arid regions of Africa, the Arabian Peninsula and remaining Asia sub-tropical regions (Roll *et al.*, 2017). The altitudinal range of samples varied from sea level up to 3.718 m above sea level with most records below 500 m (mean = 367.7 m, median = 74 m), which reflects the prevalent altitudinal range for Squamates (Buckley *et al.*, 2008, 2012). The dataset contains representatives of 34 families but only 2.71 % of the total species diversity (Figure 2B). Given the contribution of different families to the total species richness of Squamate reptiles worldwide (Uetz & Etzold, 1996; Roll *et al.*, 2017), there is an “over-representation” of species from Lacertidae, Phrynosomatidae, Teiidae, Diplodactylidae, Sphaerodactylidae and Viperidae and an “under-representation” of species from Gekkonidae, Gymnophthalmidae, Elapidae, Scincidae and Colubridae.

In addition to heterogeneity in sampling, measurements conditions and laboratory methods, calculations of water loss rates vary between studies precluding the selection of a single uniform protocol. In particular, EWL rates are reported on three different scales, namely as mass-relative values (% of initial body mass or mg per g per hour, 1542 records), absolute values (mg water per hour, 715 records), or surface-relative values (mg per cm<sup>2</sup> per hour, 269 records). To convert all EWL records to a single scale (mg per hour), we gathered records reported on different scales and used a statistical procedure to fit a calibration function to convert data from relative to absolute scale (see Supplementary File 1). Using this approach, we calculated 1880 unique estimates of absolute total EWL rates, including TEWL, CWL and RWL components for 302 species.

Preliminary non-phylogenetic analyses indicated that TEWL and CWL rates scale allometrically with body mass with an exponent of ca. 0.6-0.7 (linear regression on a log-log scale, TEWL: slope =  $0.66 \pm 0.0154$  SE, CWL: slope =  $0.59 \pm 0.048$  SE), which is close to the 2/3 allometric exponent predicted if TEWL and CWL increase linearly with surface area (see Figure 2C). The RWL component is generally smaller than TEWL and CWL components in small-sized species and increases faster with body mass (linear regression on a log-log scale, RWL: slope =  $0.88 \pm 0.058$  SE), as expected from the 3/4 predicted allometric exponent of ventilation and metabolism. After correction for body mass, records of TEWL showed no clear altitudinal cline across all samples (linear regression,  $F_{1,955} = 2.98$ ,  $P = 0.09$ ), but a small negative latitudinal cline (linear regression,  $F_{1,956} = 5.76$ ,  $P = 0.02$ ).

**Figure 2.** **A.** Geographic distribution of data records with exact coordinates of sampling location when available. The geographic location of data records is mapped over a raster map of the Global Aridity Index (GAI) for the 1970-2000 period (Trabucco & Zomer, 2019). The aridity index represents the ratio between rainfall and a measure of potential evapo-transpiration (hyper-arid:  $GAI < 0.03$ ; arid:  $GAI < 0.2$ ; semi-arid:  $2 < GAI < 0.5$ ; dry sub-humid:  $0.5 < GAI < 0.65$ ; humid:  $GAI > 0.65$ ). **B.** Phylogenetic tree of the Squamate reptiles families according to a recent time-calibrated phylogeny of Squamate species by (Zheng & Wiens, 2016). We calculated the proportion of species sampled (black portion of each barplot) according to the Reptile Database (Uetz & Etzold, 1996) and the total number of records for each family. Data deficient families are highlighted in blue. **C.** Allometric scaling of water loss rates (mg water loss per hour) with body mass in 1485 records across 305 species for which TEWL, total CWL and total RWL could be calculated. All individual data points are displayed on a log-log scale together with the best linear non-phylogenetic regression line for each EWL component.

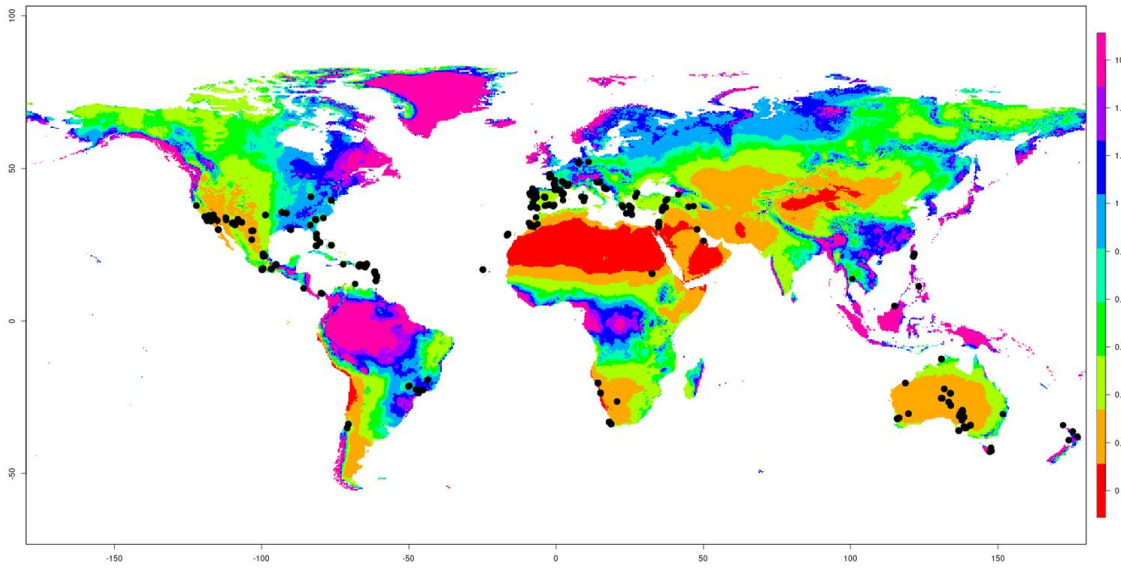


Figure 2A

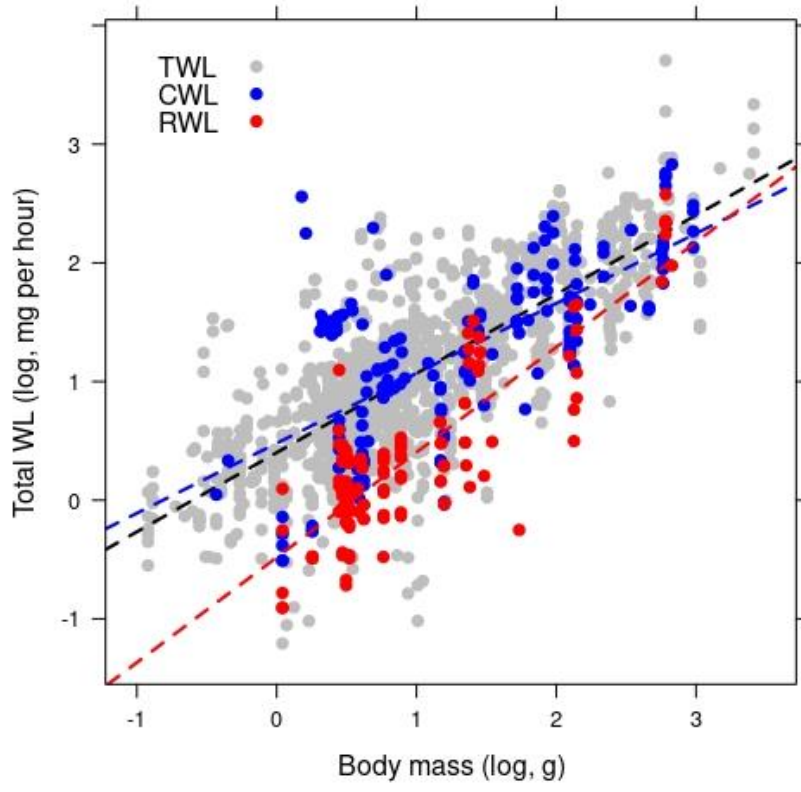


Figure 2C

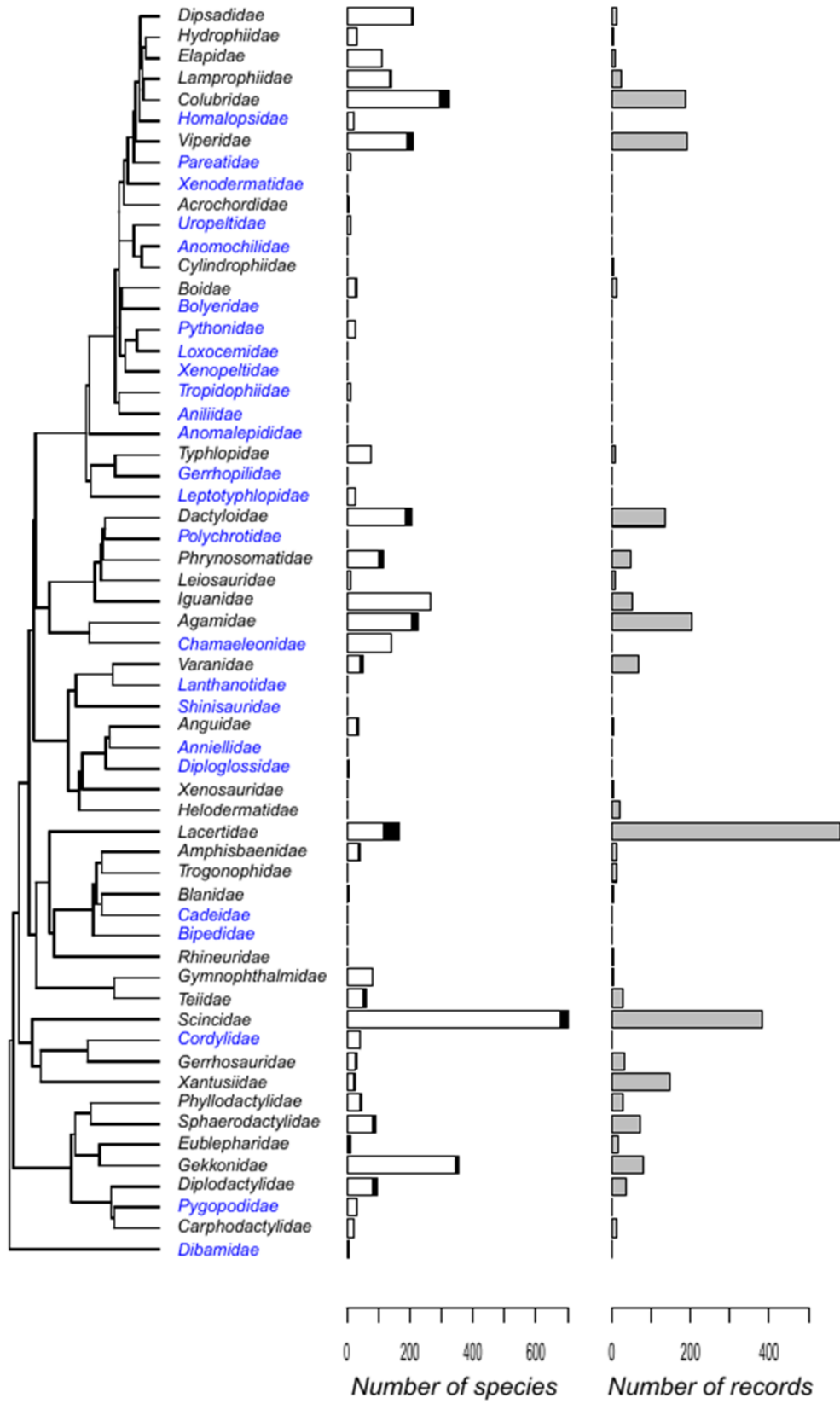


Figure 2B



#### 4. Conclusion and perspectives

The present dataset greatly expands previous compilations of EWL in Squamate reptiles by nearly doubling the number of species (ca. 100 species in Mautz, 1982; 139 species in Cox & Cox, 2015). It also provides exhaustive metadata about methods, contexts and protocols in which each unique data point was obtained. This expanded and fully annotated dataset will facilitate transparent and reproducible statistical manipulation of EWL data for future studies, allow to exam of how much variation in EWL is caused by methodological factors instead of ecological or evolutionary drivers, and will ease comparative analyses of EWL. In particular, the dataset makes it possible to calculate estimates of TEWL, CWL and RWL, to convert between different measurement scales and to identify records performed under standard conditions with non-manipulated animals at rest. This is particularly important given the substantial differences in methodology among studies and the inherent variability in EWL values caused by air temperature, air humidity or air flow during measurements. Indeed, despite earlier suggestions to better standardize protocols (Mautz, 1982), the reported data cover a broad range of methods and contexts, which precludes the use of mean species values without accounting for potential methodological artifacts.

We hope that the compiled metadata information (see Figure 1B) will foster the improvement of data reporting standards. In particular, we recommend that future studies of EWL in Squamate reptiles report systematically details of animal origin, husbandry conditions before measurements, protocols and measurements conditions, and provide central tendencies and dispersion statistics on absolute scales (mg per hour) or raw data. Our dataset further identifies critical geographic and taxonomic gaps to be filled in future investigations of EWL. The limited geographic and taxonomic coverage of our dataset reflects known-biases in herpetological and ecological research (Etard *et al.*, 2020) and is not surprising for functional traits related to physiology. Given that the water biology of Squamate reptiles has been far less investigated than their thermal biology, it will crucial that a gap-filling effort be directed to the sampling of more data in poorly sampled areas and taxonomic groups. The database will be regularly updated with these new data to provide a central resource for ecological and evolutionary research on this particular animal group.

## **Funding**

Compilation of the database was funded by the Centre National de la Recherche Scientifique (CNRS), the Agence Nationale de la Recherche (Aquatherm: ANR-17-CE02-0013 to JFLG) and a doctoral grant from Ecole normale supérieure to CC.

## **Acknowledgments**

We thank colleagues who helped with data calculation by providing raw or summary data, metadata or additional information including David Chapple, Allen Cohen, Don Bradshaw, David Chapple, Pierre-André Crochet, Sebastian Kirchof, Jason Kolbe, Michaël Guillon, Shu-Ping Huang, Jason Kolbe, Amy MacLeo, Bill Mautz, Francisco Javier Muñoz Nolasco, Anil Oguz, Panayiotis Pafilis, Catarina Rato, Abderrahim S'khifa, Graham Thompson, Miguel Vences, Philip Withers and Anamarija Žagar. We thank Luis Miguel Senzano for providing unpublished data.

## **Authors contributions**

JFLG and TVD conceived the project, organized the data collection, and collected and checked data. FB, AD, RG and OL contributed to project conception and helped with data collection and management. MA, MS and DOVA contributed data and helped with data collection. CC helped with data collection, data formatting and technical validation. All authors contributed to writing based on a first version produced by JFLG.

## **Data Accessibility**

Data are available on the Global Ecology and Biogeography webpage and on Zenodo (doi: 10.5281/zenodo.3666172).

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**Table 1 Database of standard water loss data descriptors and summary statistics data extracted from published studies and raw material.**

| Column title           | Column type   | Description  |
|------------------------|---|--|
| Unique_id              | <i>Integer</i>  | Unique row identification number.  |
| Person_name            | <i>Character list</i>   | Name of the person who extracted the data (see authors list of the data paper) – provided with an acronym.   |
| Species_name           | <i>Character</i>  | Given species name, including subspecies identity, at the time of publication as given in the source publication. This is provided in the standard format Genus name + species name + subspecies names separated by the “ ” character.   |
| Species_name_ReptileDB | <i>Character</i>  | Accepted species names based on the EMBL Reptile Database as of December 2019 extraction, including sub-species name, given in the standard format Genus name + species name + subspecies names separated by the “_” character. If the species identity could not be extracted from the publication, this column was filled in with an ‘NA’. In case multiple species are pooled together, the “spp.” species name is provided.  |
| Publication_id         | <i>Text</i>   | Source publication reported as doi:10.1000/xyz123 or with the URL of the journal publication. All articles are also listed in a freely available Zotero group bibliography.  |
| Publication_year       | <i>Integer</i>  | Year of the publication  |
| Sample_type            | Factor<br><i>Laboratory housing</i><br><i>Semi-natural population</i><br><i>Wild population</i><br><i>Not specified</i> | Describes the sample type. “Laboratory housing” implies that animals from the sample were all maintained indoor in captivity during their entire life or obtained from captive-bred animals. “Semi-natural population” implies that animals were maintained outdoor in semi-natural habitats during their life. This corresponds to rare cases where animals were kept in outdoor enclosures. “Wild population” implies that animals were obtained from natural populations. If this is not specified in the manuscript, this column was filled with the “Not specified” record. |
| Locality_name          | <i>Character</i>  | Full name from the specific location of the study as specified in the source publication. Usually provided with locality name, region or district name and country name with a comma separation. Full name was extracted for wild populations (i.e., name of the origin locality) and for semi-natural populations whenever available. Sometimes, only a country or a district locality could be retrieved from the original publication. If this is not specified in the manuscript, this column was filled with an ‘NA’.   |
| Locality_latitude      | <i>Float</i>  | Decimal degree GPS latitude coordinate of the locality (extracted from the publication or obtained from the center of the average locality using Google Maps service). Provided with a minimum 3 digits accuracy whenever possible in the WGS 84 format. If this is not available, reported as ‘NA’.   |
| Locality_longitude     | <i>Float</i>  | Decimal degree GPS longitude coordinate of the locality (extracted from the publication or obtained from the center of the average locality using Google Maps service). Provided with a minimum 3 digits accuracy whenever possible in the WGS 84 format. If this is not available, reported as ‘NA’.  |

|                   |   |   |
|-------------------|---|---|
| Locality_altitude | <i>Integer</i>  | Elevation (meters above sea level) of the locality. If this is not available, this column was filled with an 'NA'.  |
| Altitude_source   | <b>Factor</b><br><i>Author</i><br><i>GPS visualizer</i><br><i>Publication</i><br><i>None</i>  | Source of altitude data. Can be the publication, an extraction based on locality GPS coordinates using GPS visualizer tool online or a data provided by the author. When no source was found, this column was filled with the "None" item.  |
| Start_day         | <i>Integer</i>  | Start date of the collection and measurements of animals when provided. This can be useful to determine season when animals were sampled and measured for example. Depending on details, this can be given with a year, month, week, or day accuracy. When this is not available (e.g., laboratory bred animals), this column was filled with an 'NA'.  |
| Start_month       | <i>Integer</i>  |   |
| Start_year        | <i>Integer</i>  |   |
| End_day           | <i>Integer</i>  | End date of the collection and measurements of animals when provided. This can be useful to determine season when animals were sampled and measured for example. Depending on details, this can be given with a year, month, week, or day accuracy. When this is not available (e.g., laboratory bred animals), this column was filled with an 'NA'.  |
| End_month         | <i>Integer</i>  |   |
| End_year          | <i>Integer</i>  |   |
| Sample_sex        | <b>Factor</b><br><i>Females</i><br><i>Males</i><br><i>Both sexes</i><br><i>Not specified</i>  | Sex of animals from the study sample for which statistics are reported. If this is not specified in the manuscript, this column was filled with 'Not specified'.  |
| Sample_age        | <b>Factor</b><br><i>Juveniles</i><br><i>Adults</i><br><i>Both age classes</i><br><i>Not specified</i>   | Age class of animals from the study sample for which statistics are reported. If this is not specified in the manuscript, this column was filled with 'Not specified'.  |
| Treatment_group   | <b>Factor</b><br><i>Controls</i><br><i>Abnormal animals</i><br><i>Acclimation manipulation</i><br><i>Activity manipulation</i><br><i>Other manipulation</i> | Sample group of the study animals to differentiate between data from non-manipulated individuals ("Controls") and data from treatment groups of manipulated individuals (other categories). The treatment type is described with a main category type; these categories might be useful to calculate effect size of manipulative studies. This column was included to facilitate removal of data from manipulated individuals for which inter-specific comparisons of water loss rates are less meaningful. |
| Treatment_type    | <i>Text</i>   | Free text providing some details about the kind of manipulation performed on sampled animals. Additional details are provided in the comments section at the end of the data set.   |
| Acclimation_time  | <i>Integer</i>  | Number of days of acclimation in the laboratory prior to measurements for field-caught animals from wild or semi-natural populations. If this is not specified in the manuscript or if animals were from a laboratory colony, this column was filled with an 'NA'.  |

|             |   |   |
|-------------|---|---|
| WL_method   | <i>Factor</i><br><i>Change in mass of a dessicant</i><br><i>Doubly labeled water</i><br><i>Flux chamber</i><br><i>Loss of body mass</i><br><i>Gaz measurement</i> | General protocol used to quantify water loss rates (see main text for more detailed description).   |
| WL_VPD      | <i>Float</i>  | Water vapor pressure deficit (VPD) of the surrounding air during water loss rate measurements when available (kPa). When temperature and relative humidity are provided, VPD was calculated with the Magnus equation from Alduchov & Eskridge (1996). The relative humidity is assumed to be 10% when air was dried with silica gel or another chemical in a flow-through system and no exact humidity value is reported in the manuscript. Additional information about the calculation are provided as free comments in the last column of the dataset whenever necessary. Provided with at least three digits accuracy whenever possible. If this cannot be calculated, this column was filled with an 'NA'. |
| WL_TP       | <i>Float</i>  | Temperature of the surrounding air during water loss rate measurements when available (mean value or midpoint temperature from the given range, °C). Provided with at least one digit accuracy whenever possible. If this is not specified in the manuscript, this column was filled with an 'NA'.  |
| WL_flow     | <i>Integer</i>  | Air flow in ml per min during water loss rate measurements when available. If this is not specified in the manuscript, this column was filled with an 'NA'. The air flow is assumed to be zero for measurements performed in sealed or semi-sealed boxes.   |
| WL_duration | <i>Float</i>  | Duration of the water loss measurements in number of hours. Provided with at least two digits accuracy whenever possible. If this is not specified in the source, this column was filled with an 'NA'.  |
| WL_activity | <i>Factor</i><br><i>Yes</i><br><i>No</i><br><i>Not specified</i>  | A variable describing if animals were active or not during the water loss measurements. Retrieved when the original publication specifies that animals were active during measurements ("Yes") or were not active or minimally active during measurements ("No"). Otherwise, when there is no detailed information on animal activity from the protocols or data, this column was filled with the "Not specified" item.   |
| WL_Other    | <i>Text</i>   | Free text providing other useful characteristics about the protocol (acclimation type, apparatus, etc.)   |

|                 |   |   |
|-----------------|---|---|
| Sample_id       | <i>Character</i>  | Unique identifier of the study sample. This identifier is used to identify records from the same group of individuals obtained in different conditions or reported with different units. The unique identifier is given in the standard format Name first author + Abbreviated journal name + Year + Identification-Number separated with the “_” character. When there were small differences in sample size for statistics of the group of animals due to a few missing data or some outliers, we decided to give the same identifier for all statistics assuming that data were collected in more or less the same group of animals. |
| WL_component    | <i>Factor</i><br><i>TEWL</i><br><i>RWL</i><br><i>CWL</i>  | A variable describing the component of water loss measured in the sample with this protocol, which includes only 3 possibilities here: total evaporative water loss (TEWL), respiratory water loss (RWL) and cutaneous water loss (CWL, see main text for more detailed description).   |
| WL_unit         | <i>Factor</i><br><i>mg/h</i><br><i>mg/g/h</i><br><i>%/h</i><br><i>mg per cm2 per hour</i><br><i>%</i> | A variable describing the unit of the water loss rates as provided in the original publication, which includes four possibilities: absolute water loss in mg water per hour (mg/h), relative water loss in mg water per g animal per hour (mg/g/h), relative proportional water loss in % initial mass per hour (%/h) and surface specific water loss in mg water per cm <sup>2</sup> body surface (mg/cm <sup>2</sup> /h). One record was reported in % without time unit.   |
| Duplicate_check | <i>Factor</i><br><i>Keep</i><br><i>Remove</i>   | Factor describing if the sample record is a duplicate from the same sample in the same unit (this is the case of data recorded each hour during a 12hours test from few protocol also the case of records obtained from different central statistics such as median, mean or mean of the lowest values). The “Keep” factor category can be used to identify unique data records. Details are generally provided in the free text comment section when value is “Remove”.  |
| WL_mean         | <i>Float</i>  | The central statistics (mean) of the water loss rate from the sample expressed with a minimum of three digits accuracy whenever possible.   |

|                  |   |   |
|------------------|---|---|
| WL_mean_type     | <i>Factor</i><br><i>Dataset</i><br><i>Table</i><br><i>Figure</i><br><i>Text</i><br><i>Other</i> | A variable describing how mean statistics were extracted from the source publication, which includes four possibilities: direct extraction from raw data provided by the source publication or obtained from authors and/or collaborators (“Dataset”), direct extraction from a table (“Table”), direct extraction from a figure (“Figure”), direct extraction from the text (“Text”) or other techniques (e.g., statistical modeling of the data, calculation from sum of respiratory and cutaneous water loss; see also details in the comments column) |
| WL_error         | <i>Float</i>  | A measure of the dispersion (error around the mean) of WL values with a minimum of three digits accuracy whenever possible. When this cannot be extracted or when sample size equals 1, this column was filled with an ‘NA’.  |
| WL_error_type    | <i>Factor</i><br><i>SD</i><br><i>SE</i><br><i>Range</i>   | A variable describing the kind of dispersion statistics reported in the source, which includes three possibilities: sample standard deviation (SD), standard error (standard deviation of the mean, SE) or range (difference between maximum and minimum values). When this cannot be extracted or when sample size equals 1, this column was filled with an ‘NA’.  |
| WL_error_details | <i>Factor</i><br><i>Sample error</i><br><i>Error in a statistical model</i>                     | A variable describing if the dispersion statistics is calculated from sample statistics (Sample error) or using a statistical model from the variance components.   |
| WL_N             | <i>Integer</i>  | Sample size for the WL statistics (total number of individuals in the sample). When this cannot be extracted, this column was filled with ‘NA’.   |
| SVL_mean         | <i>Float</i>  | Mean snout to vent length of animals from the sample (mm) provided with a maximum of two digits. When this cannot be extracted, this column was filled with ‘NA’.   |
| TL_mean          | <i>Float</i>  | Mean total length of animals from the sample (mm) provided with a maximum of two digits. When this cannot be extracted, this column was filled with ‘NA’.   |

|               |   |  |
|---------------|---|--|
| Shape_score   | <i>Factor</i><br><i>Juvenile lizard</i><br><i>Juvenile snake</i><br><i>Lizard</i><br><i>Snake</i><br><i>Worm lizard</i> | Factor describing the general shape of the animals, essentially to make test checks of the size, length and mass data.   |
| BM_mean       | <i>Float</i>  | Mean body mass of animals from the sample (g) provided with a maximum of two digits. When this cannot be extracted, this column was filled with 'NA'.  |
| BM_error      | <i>Float</i>  | A measure of the dispersion of body mass values with a minimum of two digits accuracy whenever possible. When this cannot be extracted or when sample size equals 1, this column was filled with an 'NA'.  |
| BM_error_type | <i>Character list</i><br><i>SD</i><br><i>SE</i><br><i>Range</i>   | A variable describing the kind of dispersion statistics reported for body mass, which includes three possibilities: sample standard deviation (SD), standard error (standard deviation of the mean, SE) or range (difference between maximum and minimum values). When this cannot be extracted or when sample size equals 1, this column was filled with an 'NA'.   |
| SA_mean       | <i>Float</i>  | Mean surface area of animals (cm <sup>2</sup> ) provided with a maximum of two digits. When this cannot be extracted, this column was filled with 'NA'.  |
| Quality_code  | <i>Character list</i>   | A complex categorical variable allowing to rank data quality using a set of comments separated by commas. First comment describes data quality and extraction mode. Second and third comments describe the kinds of abnormality in the data, which may refer to the condition and state of the animals, the test conditions or some kind of pre-measurement manipulative protocol. Regarding quality, it is a subjective score with "high" (good methodology, metadata are all reported and statistics are provided with enough details), "low" (poor methodology, conditions are not reported and statistics are poorly reported) and "medium" (one of the critical aspect of a "high" score is missing). Further details are provided in the Comments column to justify further the study quality and explain environmental conditions before and during measurements. |

|                 |   |   |
|-----------------|---|---|
| Study_standards | <i>Character list</i><br><i>Low quality</i><br><i>Medium quality</i><br><i>High quality</i> | A simple categorical variable describing the quality of the data (low, medium, or high scores from the Quality_code). This is useful for future analysis of the data to select a subset of the data set with the best standards.  |
| Data_standards  | <i>Character list</i><br><i>Usable data</i><br><i>Not usable data</i>                       | A simple categorical variable describing if the data can be used to compare standard water loss rates within and between species. Further details are provided in the Comments column to justify why a data record should not be used. This is useful for future analysis of the data to select a subset of the data set with the best standards. |
| All_comments    | <i>Text</i>   | Free text giving detailed contextual information about the methods and data compilation.  |

## References

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## Supplementary File 1: Terminology and methods to quantify EWL

Experimental methods, confounding methodological factors and sub-components of water loss have been discussed in earlier syntheses on osmoregulation and hydroregulation in reptiles (Bentley & Schmidt-Nielsen, 1966; Shoemaker & Nagy, 1977; Mautz, 1982; Bradshaw, 1997) and are also summarized in a recent mechanistic review of water loss for lizards, which is also relevant to hydroregulation for snakes and amphisbenians (Pirtle *et al.*, 2019). Total water loss (TWL) can be defined as the total amount of water lost per unit time by an animal, and it must be balanced by water input from food, water input from drinking water and metabolic water production. Because reptiles are slow metabolism, ectothermic animals, metabolic water production is generally considered negligible in these species compared to water intake from food and drinking. Although these animals generally lack distinct water stores, some lizard species can use the urinary bladder as a physiological reservoir (Davis & DeNardo, 2007) and some viviparous snakes can also utilize bounded water of muscular tissues to compensate for water loss under challenging conditions (Brusch *et al.*, 2018). Thus, we cannot exclude that some squamate reptile species may rely on internal stores of water even if that is not the general rule.

Components of total evaporative water loss (TEWL) include (1) cutaneous evaporative water loss (CWL) where body water is generally lost by convection or diffusion from the body surface to the air through a boundary layer and (2) respiratory evaporative water loss (RWL) where water loss is a direct consequence of gas exchange for respiration in the lung (Mautz, 1980, 1982). Sub-components of RWL include standard ventilation associated with respiration at rest or during field activity, which is proportional to metabolic rates, body temperature, and oxygen extraction efficiency (Pirtle *et al.*, 2019), and panting, a behavior generally initiated above a temperature (called the panting threshold) and involved in thermoregulation and hydroregulation when animals are exposed to a heat stress (Loughran & Wolf, 2020). The CWL includes at least three different sub-components namely trans-epidermal evaporative water loss through the skin, evaporative ocular water loss across the surface of the eyes (Pirtle *et al.*, 2019), and cloacal evaporative water loss (DeNardo *et al.*, 2004). Most studies do not allow to separate these three sub-components accurately and the relative importance of each sub-component is still under active investigation. Cutaneous water loss depends primarily on micro-climatic environmental conditions (temperature, air humidity, and wind speed especially), behaviors (in laboratory conditions, primarily activity and position of the animal), surface and shape, and



skin resistance to water loss ( $R_s$ ), the best standardized measure of cutaneous resistance to water loss to compare data across samples, populations and species because it depends only on skin properties (Gates, 1980; Mautz, 1980). Several studies have quantified and compared  $R_s$  among closely related squamate reptile species (Dmi'el, 1998, 2001; Oufiero & Van Sant, 2018), but accurate values of  $R_s$  are generally unavailable for most species to date (Mautz, 1982). In addition, biases in the calculations of  $R_s$  are much stronger than biases in calculations of water loss rates because accurate calculation of  $R_s$  rely on physical models or empirical methods that account for the detailed evaporation processes (convection and diffusion) to correct for effects temperature conditions, air boundary layer and measurement apparatus on CWL (e.g., Riddell *et al.*, 2017). For all these reasons, global comparative analyses of resistance to water loss rely on measurements of EWL rates rather than  $R_s$  (Cox *et al.*, 2003). Yet, techniques have been proposed to calculate *a posteriori*  $R_s$  from measurements of TEWL and RWL in controlled conditions (Kearney & Porter, 2004; Pirtle *et al.*, 2019).

Typical methods to measure total evaporative water loss (TEWL) must exclude body water lost from urine and feces, which imply either (1) record of water loss for animals maintained in controlled laboratory or semi-natural conditions, deprived of food and water and in a post-absorptive state or (2) record of the water budget of animals together with separate estimates of water output from feces and urine (Mautz, 1982; Bradshaw, 1997). The later technique has been used for field-active animals using doubly-labeled water to measure the average field metabolic rate and water influx over a period of time (Nagy & Peterson, 1988). We included data records of water loss based on this method (Gans *et al.*, 1968; Congdon *et al.*, 1979; Grenot *et al.*, 1987) but all were performed under non-standard conditions with active animals and generally report TWL instead of TEWL. They were therefore considered not usable for comparative analyses even if we report them in the database. The former technique includes four distinct methods to quantify standard EWL rates, which can be defined as the “evaporative water loss rates of animals at rest, in a post-absorptive state and under controlled temperature conditions”.

The first, simplest technique involves repeated measurements of body mass to quantify body mass loss due to water loss over a given period of time, generally a few hours, under controlled, desiccating conditions and generally in still air (e.g., Moen *et al.*, 2005; Sannolo *et al.*, 2018). The second technique involves measurement of the mass increase of a dessicant after exposure to excurrent air from a chamber at controlled humidity and temperature where the animal is resting (e.g., Duvdevani & Borut, 1974; Zucker, 1980; Lahav & Dmi'El, 1996). The

third technique involves continuous measurement of water vapor from incurrent and excurrent air of a flow-through chamber, generally together with measurements of standard metabolic rates (e.g., Reichling, 1957; Withers, 1993; Dupoué *et al.*, 2015). Independent estimates of CWL and EWL can be obtained with the two later techniques provided gas exchange from ventilation can be separated from skin evaporation (Mautz, 1982). The last, more recent technique relies on flux chambers to quantify trans-epidermal water loss (often to as TEWL) from a specific skin area and includes condenser-chamber or unventilated-chamber instruments (e.g., Lillywhite *et al.*, 2009; Guillon *et al.*, 2014). All techniques rely on assumptions that animals do not defecate or urinate, remain inactive and do not pant during the tests. The first can be improved by excluding records with feces or urine and removing abnormal records, and we generally considered that this was the case in reported data except when specifically mentioned in the study. The second is more difficult to ensure and usually requires to test animals at night or in the night, at low ambient temperatures and with minimum disturbance or to record activity patterns, which is rarely done. The third is easily reached unless the species exhibit a panting behavior and the test temperature is close to or above the panting threshold, which is generally close to 40°C and the maximum critical temperature threshold. Whenever one of these three conditions was not reached, we consider that the protocol did not measure accurately the standard EWL and therefore classified the data standard as “Not usable” in the dataset.

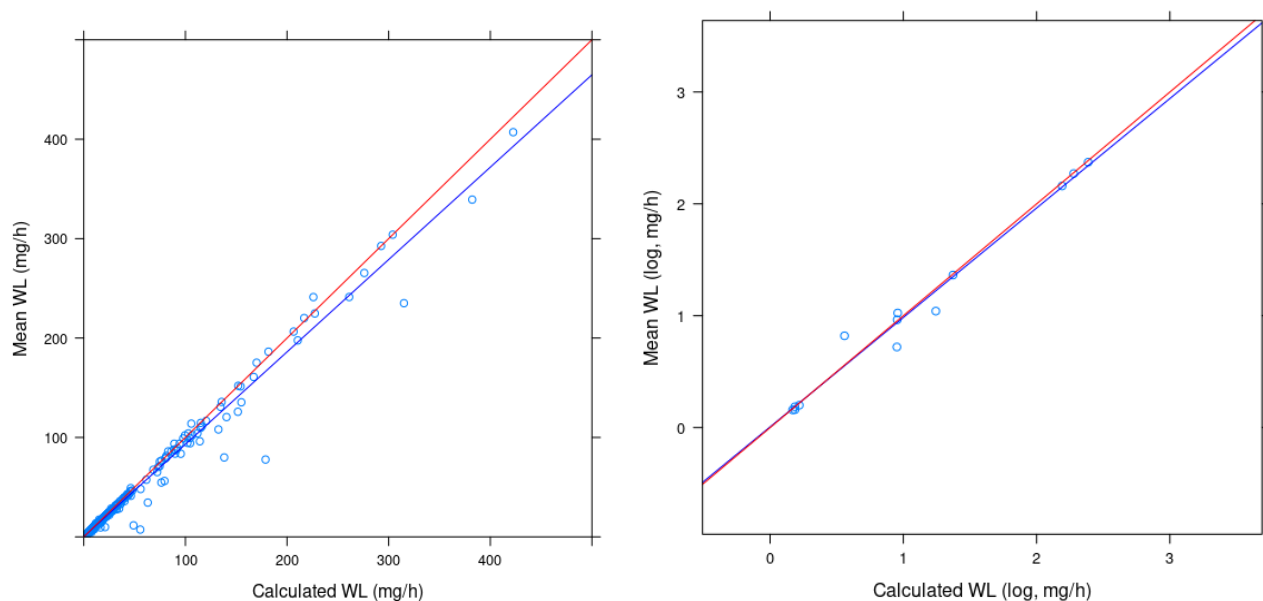
Most EWL records were provided in different units with about xxx % of TEWL, xxx % of the CWL and xx % of the RWL records provided in absolute unit (mg water per hour) instead of relative units (% mass per hour, mg per g mass per hour or mg per cm<sup>2</sup> per hour) calculated by dividing EWL with body mass or surface area. In addition, surface area was generally calculated and not measured; for example, species-specific regression curves between body mass and body area (instead of direct surface measurements) were used to convert size from mass to surface units. When EWL is provided in relative units (for example, mg water lost per hour per g mass), it may be tempting to convert the data into absolute unit using a simple Rule of Three (for example, multiplying the mean relative record by mean body mass of the sample) but this rule ignores that the expectation of a product of random variables depends also on the covariance between the two random variables. There is good evidence that relative values of EWL scale with body mass or surface area both within and between species (Green, 1969; Mautz, 1980). In order to transform the records of the ReptEWL dataset from relative units to absolute units, we extracted xxx duplicated records for the same sample of animals in the same experimental conditions provided in mg per hour and in at least one relative unit. We then used

mean water loss and mean body mass (or mean surface area) to convert data from the relative unit to the absolute unit using the Rule of Three calculation. We next compared the predicted water loss rates in the converted, absolute unit with the reported, original value in this unit provided in the source publication for this same record using a single major axis regression (Warton *et al.*, 2012). Absolute water loss rates in mg per hour calculated from mass-relative or surface-relative mean values using the Rule of Three are generally strongly correlated with original values (see Figure S1 below), except when records are collected in highly heterogeneous data-sets with large variation in body mass among individuals (e.g., Guillon *et al.*, 2014).

## Figure S1

Figure S1A. Calibration with mass-relative values

Figure S1B. Calibration with surface-relative values



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## **Chapitre 2) Climate aridity and habitat drive geographical variation in morphology and thermo-hydroregulation strategies of a widespread lizard species**

*Published in Biological Journal of the Linnean Society in 2022*

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Running title: Ecophysiological variation in lizard

## Abstract

Thermo-hydroregulation strategies involve concurrent changes in functional traits related to energy and water balance as well as thermoregulation and play a key role in determining life history traits and population demography of terrestrial ectotherms. Local thermal and hydric conditions should be important drivers of the geographic variation of thermo-hydroregulation strategies but we lack studies that examine these changes across climatic gradients in different habitat types. Here, we investigated intraspecific variation of morphology and thermo-hydroregulation traits in the widespread European common lizard (*Zootoca vivipara lousiantzi*) across a multidimensional environmental gradient involving independent variation in air temperature, rainfall and differences in habitat features (access to free standing water and forest cover). We sampled adult males for morphology, resting metabolic rate, total evaporative water loss and cutaneous evaporative water loss (EWL) and thermal preferences in 15 populations from the rear to the leading edge of the distribution across an altitudinal gradient ranging from sea level to 1750 m. Besides a decrease in adult body size with increasing environmental temperatures, we found little effects of thermal conditions on thermo-hydroregulation strategies. In particular, relict, lowland populations from the warm rear edge showed no specific eco-physiological adaptations. Instead, body mass, body condition and resting metabolic rate were positively associated with a rainfall gradient, while forest cover and water access in the habitat through the season further influenced EWL. Our study thus emphasizes the importance of rainfall and habitat features rather than thermal conditions on geographic variation in lizard morphology and physiology.

Keywords: aridity - evaporative water loss – lizards – metabolism – morphology - thermal preferences - reptiles

## 1. Introduction

Ongoing climate change dramatically impacts biodiversity and increasing our ability to predict future ecological effects of climate changes is one of the main challenges facing research in ecology today (Bellard et al., 2012). In widely distributed organisms, gradient analyses of intraspecific variation along contrasted climate conditions can inform on the environmental sensitivity and capacity of different species to adapt to future changes (Blois et al., 2013). Terrestrial ectotherms are sensitive to environmental temperatures and highly vulnerable to global warming, especially to heat and drought stress that impairs activity, causes physiological stress and can reduce fitness and population growth (Huey et al., 2009, 2012; Sinervo et al., 2010; Kubisch, Fernández, & Ibarzüengoytía, 2016).

Terrestrial ectotherms can cope with heat and drought risks through changes in thermoregulation (i.e., heat exchange and body temperature regulation) and hydroregulation traits (i.e., water balance regulation), collectively referred to as their “thermo-hydroregulation” strategies (Rozen-Rechels et al., 2019). Yet, large uncertainties remain about the respective roles of physiological and behavioral response and the geographic variability of body temperature and water balance regulation. Behavioral and physiological maintenance of an optimal body temperature and hydration state depends to a large extent on spatio-temporal variability in operative temperatures, water availability and water vapor deficit (Sears et al., 2019; Rozen-Rechels et al., 2019).

Historically, comparative physiology research has however focused on the thermal biology of terrestrial ectotherms (Huey et al., 2012; Sunday et al., 2014; Seebacher, White, & Franklin, 2015; Artacho et al., 2017; Caldwell, While, & Wapstra, 2017; Rutschmann et al., 2020). For example, populations of the same species of lizards can display different critical thermal limits depending on local thermal conditions, particularly those characterizing the cold end of the thermal performance curves (Pontes-da-Silva et al., 2018; Herrando-Pérez et al., 2020; Bodensteiner et al., 2021). Another general finding is the metabolic cold adaptation in energy expenditure where organisms from cooler climates have higher basal metabolic rates, probably to partly compensate for the slow development and pace-of-life associated with low environmental temperatures in ectotherms (Seebacher, 2005; Pettersen, 2020; Dupoué et al. 2017a). Instead, intraspecific studies on thermal preferences (i.e., preferred body temperatures in the absence of thermoregulation costs) have led to inconclusive results, with some authors demonstrating geographic differences in thermal preferences (Trochet et al., 2018; Rozen-



Rechels et al., 2021) whereas others suggest that thermal preferences are more evolutionarily rigid than other thermal biology traits (Clusella-Trullas & Chown, 2014). In addition, we lack knowledge on how intraspecific variations correspond with the observed variation between species.

Heretofore, most studies of geographic variation in reptile thermal traits have focused on thermal clines such as broad altitudinal or latitudinal gradients in air temperatures (Gvoždík & Castilla, 2001; Zamora-Camacho, 2013; Osojnik et al., 2013; Clusella-Trullas & Chown, 2014; Trochet et al., 2018; Plasman et al., 2020). Such broad thermal clines usually confound with variation in precipitation, making the two factors difficult to distinguish. Yet, habitat characteristics can amplify or buffer thermal variability and recent works have highlighted the importance of water availability and microclimatic conditions when studying responses of terrestrial ectotherms to environmental modifications (Wegener, Gartner, & Losos, 2014; Miller & Lutterschmidt, 2014; Chiacchio et al., 2020). In squamate reptiles, availability of water and suitable microhabitats are essential for optimal thermoregulation and hydroregulation, as behavioral exploitation of microhabitats (such as wet shelters from the vegetation) can buffer these species from the deleterious effects of a temperature increase or a rainfall reduction (Sears et al., 2016; Rozen-Rechels et al., 2020). In addition, the costs of maintaining high body temperature in heliothermic species usually increase when those species face a reduction of water availability in the environment, leading to the selection of suboptimal body temperature (Rozen-Rechels et al., 2020). Variation in morphological traits can also be strongly affected by water availability and microhabitat features (Ashton, 2001; Olalla-Tárraga et al., 2009, Roitberg et al., 2020). Teasing apart the effects of thermal gradients, water availability and local habitats on thermo-hydroregulation strategies and morphology requires comparative analyses along independent clines of ambient temperature and rainfall in sites with contrasted microhabitat features.

Another limitation is that comparative studies of ectotherm hydroregulation strategies are still lagging behind those of thermoregulation (Pirtle, Tracy, & Kearney, 2019). Hydroregulation involves both behavioral and physiological mechanisms such as behavioral activity and shelter use, behavioral microhabitat selection, or physiological mechanisms of evaporative water loss through the skin (Pirtle et al., 2019). Skin resistance to evaporative water loss is primarily determined by the presence of a lipid layer in the epidermis of lizards and snakes, which acts as a physical barrier to water loss and determines total evaporative water loss (TEWL) rates (Roberts & Lillywhite, 1980). Plastic changes in TEWL rates over a few weeks or months have

been demonstrated in some lizards and snakes when individuals are exposed to different air moisture or water availability conditions (Kobayashi, Mautz, & Nagy, 1983; Moen, Winne, & Reed, 2005). Phylogenetic analyses of TEWL in Lacertid lizards also indicate that this trait is more evolutionarily labile and more variable than thermal preferences, and evolves towards lower values in warmer environments (Garcia-Porta et al., 2019). Yet, studies of co-variation in TEWL and thermoregulation traits are rare, and those suggest that the two sets of functional traits may often vary independently from each other (Sannolo et al., 2020; S'khifa et al., 2020).

Here, we performed such a comparative study in the Lacertid *Zootoca vivipara lousilantzi* (Arribas, 2009), which is a ground-dwelling lizard with an oviparous reproductive mode. We examined variation in thermoregulation and hydroregulation traits across a geographic gradient in South-Western France from relict populations located at the hot distribution margin to populations located at the colonization front in cold, highland habitats (Dupoué et al., 2021). Using an integrative approach, we quantified geographic variation in (1) thermal preferences ( $T_{\text{pref}}$ ), (2) hydroregulation physiology (total evaporative water loss TEWL and its cutaneous subcomponent CEWL), (3) energy metabolism (resting metabolic rate) and (4) morphology (body size, body surface, body condition and caudal reserve) of adult male lizards from fifteen populations. We also evaluated physiological dehydration through osmolality to investigate its correlation with climate conditions and habitat. To avoid confounding effects of inter-individual differences due to age, sex or seasonality, we sampled only sexually mature males during the reproductive season. We further compared our studied populations with those of two taxonomic outgroups sampled with the same technique, including two nearby French populations of the closely related viviparous reproductive mode *Z. vivipara vivipara* and one population of the distantly related wall lizard *Podarcis muralis* from semi-mesic, saxicolous environments (Garcia-Porta *et al.*, 2019). This sampling design allowed us to test if hydroregulation physiology and energy metabolism traits are more flexible than thermal preferences, if those traits covary or vary independently from each other, and if intraspecific variability is more constrained than interspecific variability. We hypothesized that geographic variation is best explained by considering both local (habitat) and large-scale (climate) features.

First, populations with a restricted access to water should have lower TEWL rates and thermal preferences than those with permanent access after controlling for effects of the macrohabitat climate (Dupoué et al., 2017b; Rozen-Rechels et al., 2021).

Second, populations from more arid environments should have lower TEWL rates as seen in interspecific comparisons for reptiles (Cox & Cox, 2015; Garcia-Porta et al., 2019), but it is unclear whether thermal preferences should increase or not with macrohabitat temperature and aridity. Metabolism at rest should be lower in warmer and more arid environments, as seen in recent broad scale comparisons (Dupoué, Brischoux, & Lourdais, 2017a).

Third, we expect inter-individual covariation between functionally related traits. For example, total evaporative water loss (TEWL) being the sum of respiratory (REWL) and cutaneous evaporative water losses (CEWL), we expect a positive correlation between TEWL and CEWL measured in similar conditions in the same animals and also a correlation between TEWL and oxygen consumption ( $VO_2$ ), since REWL scales linearly with  $VO_2$  (Pirtle et al., 2019).

## **2. Material and methods**

### 2.1. Study species

The common lizard *Zootoca vivipara* is a small lacertid lizard (Reptilia: Lacertidae) with a wide Eurosiberian distribution ranging from Southern France and Central Europe to Japan on Hokkaido Island (Surget-Groba et al., 2006). It occupies cold and wet habitats including open heat lands, humid grasslands and peat bogs as well as clearances and clear-cuts surrounded by forests. Most European populations are viviparous except for some oviparous lineages distributed at the Southwestern margin of the range in France and in Southern Central Europe (Surget-Groba et al., 2001). We studied the oviparous South-West European common lizard, *Z. vivipara louislantzi*, which consists of four major subclades and inhabits a range of habitats from sea levels to highland (Milá et al., 2013). We focused our field sampling on clade B2 in France to avoid inter-population differences due to genetic differentiation between clades with potential introgression (Milá et al., 2013; Dupoué et al., 2021).

### 2.2. Sampling design and sampling site

In a recent study of the range distribution of this sub-species in South-Western France, we found that temperature, rainfall and forest cover shape demographic and genetic variability from the rear to the leading edges (Dupoué et al., 2021). We sub-sampled sites within this geographic range to select fifteen populations along a sharp climate gradient for both temperature and rainfall, and contrasted habitat features including differences in access to free-standing water

and in forest cover. These variables adequately quantify aspects of the thermal and hydric gradients that should be relevant considering previous findings showing interactive effects of hydric and thermal conditions on thermoregulation behavior (Rozen-Rechels et al., 2021). In addition, geographic variation of some morphological and reproductive traits correlates with habitat humidity and rainfall in this species (Lorenzon, Clobert, & Massot, 2001; Marquis, Massot, & Le Galliard, 2008; Dupoué et al., 2017b).

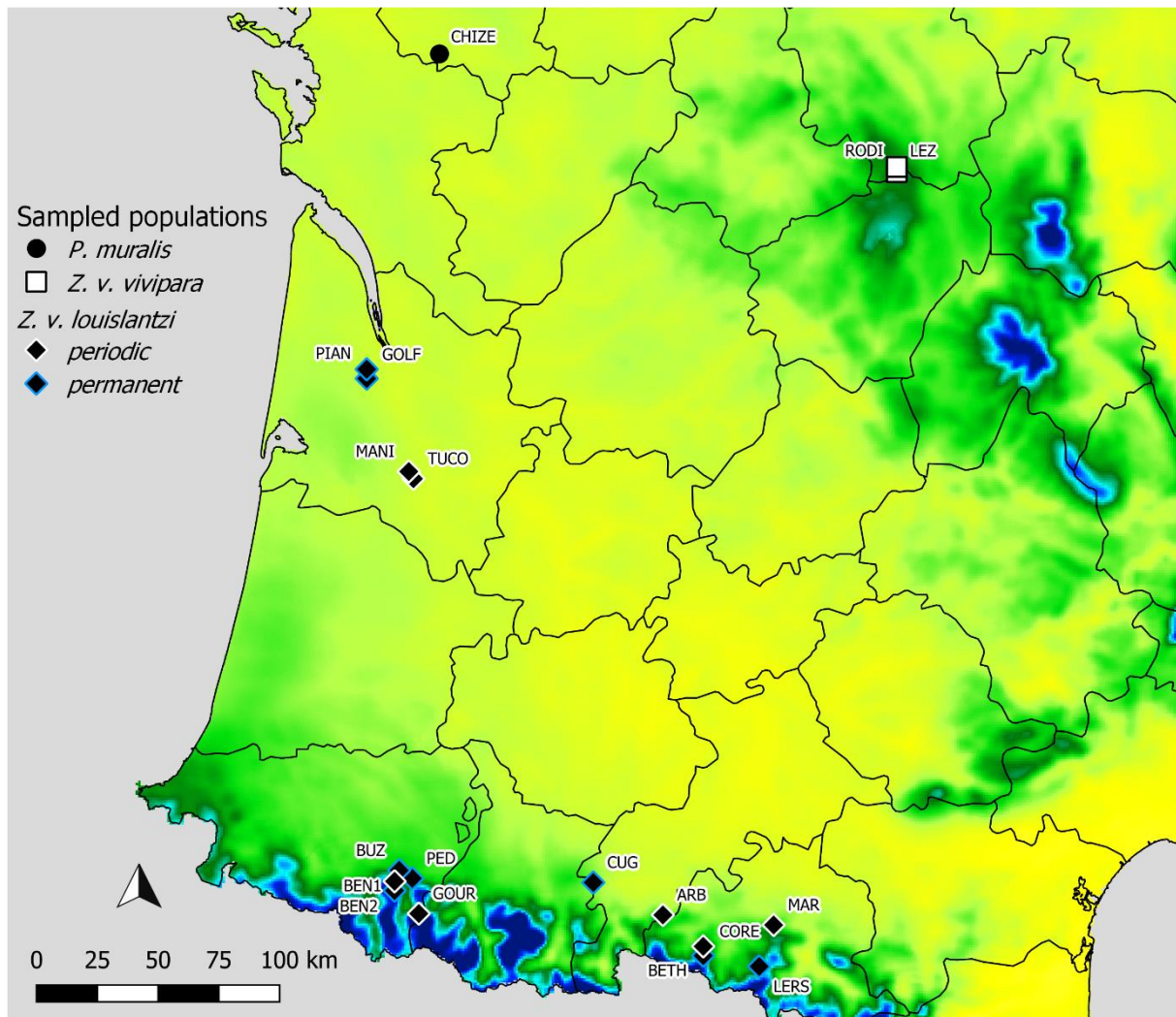
To select our focal populations, we gathered altitude, habitat data and climate information for ca. 130 known occurrence sites in South-Western France (Cistude Nature and Nature en Occitanie, unpub. data). For each site, we calculated the average temperature and the cumulative precipitation during the activity season (from April to September) using 30 Arcsec resolution climatic data derived from AURELHY climatic model outputs developed by Météo-France (averages from the 1971-2000 period of weather survey, (Canellas et al., 2014)). We also extracted the minimum temperature of the coldest month ( $T_{\text{cold}}$ ) and the maximum of the hottest month ( $T_{\text{hot}}$ ) and the annual precipitations  $P$ , to calculate the annual pluviometry quotient  $Q = (100 \times P) / (T_{\text{hot}}^2 - T_{\text{cold}}^2)$ , also called the Emberger index (Emberger, 1955). This parameter allows to discriminate mesic (high  $Q$  index) from arid (low  $Q$  index) climates.

We then selected 15 representative populations of *Z. vivipara louislantzi* (clade B2) distributed along the altitudinal gradient with different temperature (maximum temperatures during activity period ranging from 20°C to 26°C), rainfall levels (total precipitation during activity period ranging from 360mm to 850mm) and access to free standing water at the same altitude (see Supplementary Table S1 and Figure 1). Map was plot using QGIS software version 3.10.9-A Coruña with the perimeters of the French departments in the background. These populations were also associated to an altitudinal gradient (40-1750 m) and to a variety of habitats including (1) sites with permanent access to free standing water (presence of water sources available to the lizards throughout the year such as lake, streams, peat bogs) versus temporary access to free standing water (water body only present after a rain or during the wet season, see Dupoué et al., 2017b) and (2) sites with forest cover versus open habitats, calculated with a forest cover index detailed in Rutschmann et al. (2016). We chose to sample populations with as much contrasted access to water and forest cover along the altitude and aridity gradients as possible (see Figure S2), and then there was no significant correlation between mean temperature or mean rainfall and water access, but a small and significant correlation (Kruskal-Wallis test = 5,  $df = 1$ ,  $p = 0.02$ ) between temperature or rainfall and forest cover. For comparative purposes, we further sampled 2 populations of the viviparous *Z. vivipara vivipara* clade E1 from the

nearest locations in the Limousin area at ca. 800m asl (Figure 1). We also sampled wall lizards (*Podarcis muralis*) from a lowland population in the same area (Chizé population, 46°08'51.5"N 0°25'36.4"W, ca. 64 m asl), which fell within the climatic range of the common lizard populations in order to limit geographic effects. This widespread species is adapted to more arid environments and served us as an outgroup to compare with the common lizard, which is adapted to mesic and cold environments (Figure 1). We attempted to capture ca. 10 adult males per population (see Table S2, Supp.Info).

Oviparous populations from South-Western France are characterized by a strong seasonal activity pattern, a sexual maturation at the age of one to two years old, facultative multiple clutches per year, and with an adult snout-vent length (SVL) ranging from 45 to 75 mm in males (Heulin, 1987; Heulin, Osenegg-Leconte, & Michel', 1997). Sexually mature males were told apart by body coloration, presence of femoral pores, and hemipenis shape. Since sexually mature males most likely emerge earlier during the season in warmer environments, lowland populations were sampled first to reduce differences in reproductive phenology among populations (from 17th of April 2019 in the lowland populations until the 7th of May 2019 in the highland).

**Figure 1:** Map of populations sampled for *Z. vivipara louslantzi* (clade B2) in South-Western France at different pluviometry quotient levels (background colors, yellow to blue gradient: from low to high pluviometry) and with permanent (diamond with blue outline) or periodic (diamond with white outline) access to water. Two outgroup populations of *Z. vivipara vivipara* (LEZ and RODI) and one of *P. muralis* (CHIZE) were included for comparative analyses.



### 2.3. Field measurements and housing

We captured individuals by hand and brought them back to a laboratory (CEBC, CNRS, Villiers-en-bois, France) within two to four days following capture. Age class of sexually mature males was unambiguously scored using external morphology to separate young adults (aged less than one calendar year and born in 2018) from older adults (aged two or more than two calendar years). One person (JFLG) then measured SVL and total length to the nearest mm with a plastic ruler and body weight to the nearest mg with an electronic balance. The ventral and dorsal surface of all males were scanned at 600 dots per inch using a flatbed scanner

(Hewlett-Packard Co., ScanJet 3670, see Bruschi *et al.*, 2020) with animals evenly laid flat on the scanner on their belly and back using a blue foam, to measure body surface and tail width, since body area is a critical determinant of total water loss. To do so, we processed the digital images with the software ImageJ (Schneider, Rasband, & Eliceiri, 2012) using the Analyze Particles function to calculate the body area. We first determined a threshold in terms of color of pixels to tell the lizard apart from the background, and then the software automatically counted the number of pixels corresponding to the lizard body area. We repeated the analysis with ventral and dorsal scans of each lizard and summed the two areas. Tail width was further measured at the 7th, 10th and 12th subcaudal scales, and at maximum width (Bruschi *et al.*, 2020). Scans were not always exploitable for this measurement with 124 usable values out of 142 for the 10th and 12th subcaudal scales, 117 for the 7th subcaudal and 94 for the maximum width. The day of capture, we further took a blood sample from the post-orbital sinus using 1 to 2 micro-capillary tubes (ca. 20–30  $\mu$ l whole blood) and kept samples at  $\sim 4^{\circ}\text{C}$  in a cooler. We used blood sample to assess how plasma osmolality (one of the best proxies for physiological dehydration in lizards, see Peterson, 1996) vary among populations and whether lizards could maintain normosmolality or not in the face of strong variation in environmental conditions (Dupoué *et al.*, 2017b). In the laboratory, samples were centrifuged at 9500 g for 5 min to separate plasma from red blood cells. Plasma samples (ca. 5–15  $\mu$ l) were immediately frozen at  $-28^{\circ}\text{C}$  until osmolality assays. Later, plasma osmolality was determined using a vapor pressure osmometer (model Vapro 5600, ELITechGroup) with the protocol described in Wright, Jackson, & DeNardo (2013) and adjusted to small plasma volumes (Dupoué *et al.*, 2017b). Before analyses, plasma was diluted (1:3) in standard saline solution (Osmolarity = 280  $\text{mOsm.l}^{-1}$ ) to obtain 2 duplicates per sample. Thus, we were able to estimate an intra-assay coefficient of variation ( $\text{CV} < 3\%$ ).

Animals were kept separated in individual terraria (35 $\times$ 25 $\times$ 12.5cm) with peat soil, a PVC tube for shelter and a water cup with permanent access to free standing water. During captivity, we provided all lizards with the same thermal gradient from 20 to 34 $^{\circ}\text{C}$  for 8h per day (09:00-17:00) using heating wires placed under one end of each terrarium. Lizards were all fed with live crickets (*Acheta domesticus*) every two days and had water sprayed 3 times a day in addition to free water available *ad libitum*. Physiological assays and behavioral trials started after a one-week acclimation period (see below). Once all measurements were completed, all males were released at capture location.

#### 2.4. Ecophysiological measurements

We separated animals from different populations randomly in 12 lots of 15 lizards. Then, each lot went through the same succession of laboratory measurements as summarized in Figure S1 (Supp.Info). Individuals were maintained without food for 4 days and weighed with a high precision scale ( $BM \pm 0.01\text{mg}$ ) at 8 pm at the end of Day 3. They were then placed overnight in an open-top plastic box with no water nor food inside a climatic chamber set at 20°C and 60% relative humidity. All lizards were re-weighed at 8 am the following day (Day 4) and moved back to their individual terrarium. We assessed total evaporative water loss (TEWL) from the loss of mass in mg per hour after removing individuals having defecated during the night (N=27). In squamate reptiles, body mass loss reflects TEWL (i.e., the sum of ventilatory and cutaneous evaporative water losses) in resting animals because variation in body mass is mostly due to water loss (Dupoué et al., 2015; Moen et al., 2005). We further measured body mass loss at rest during metabolic rate assays in Day 6 (Figure S1) and found a strong correlation between these two measurements of TEWL, showing that this protocol was highly repeatable (Repeatability estimation using the linear mixed-effects model method including animals that did not defecate during the tests,  $N = 59$ ,  $R = 0.64 \pm 0.08$ ,  $p < 0.0001$ ).

The next day (Day 5), we measured preferred body temperatures  $T_{\text{pref}}$  of fasted lizards in a photothermal gradient inside a neutral arena where water was available *ad libitum* and lizards were safe from predators and competitors (Artacho, Jouanneau, & Le Galliard, 2013). Around noon, lizards were placed individually in a 60 cm-long plastic box with a 40W light bulb at one end and a water cup (Trochet et al., 2018). By maintaining the air of the room at 18°C, we could simulate a thermal gradient ranging from 18°C to 43°C below the light bulb. Experiments started around 1pm and we collected data after at least a 30 min long acclimation period. Every 20 min during 3h30, we measured the surface temperature of the lizard on the back with a high precision infrared thermometer (Raytek, Raynger MX2). We collected 11 repeated measurements for each individual to assess thermal preference ( $T_{\text{pref}}$ ) values. Differences in thermal preferences between populations were then analyzed using all observations except those of animals buried in the soil, which we considered irrelevant because lizards were “inactive”. At the end of the day, we also measured the core body temperature of 120 individuals (randomly chosen among types and populations) with the tip of a K-type temperature probe (Hanna HI 935002, Hanna Instruments) inserted in the cloaca. This allowed us to calculate a calibration curve between surface temperature and core body temperature. We



found a highly significant, close to 1:1 relationship between core body temperature and surface infrared measurements for the 3 taxonomic groups of lizards (major axis regression forced to 0, slope =  $0.96 \pm 0.01$ ,  $p < 0.0001$ ,  $R^2 > 0.99$ ). We used this regression to convert all surface temperatures to core body temperatures for further analyzes.

At Day 6, we used the indirect calorimetric method with a closed respirometer system described in (Foucart et al., 2014) to measure resting metabolic rate from the CO<sub>2</sub> and O<sub>2</sub> gas exchanges between animals and the atmosphere. Lizards fasted for 6 days were weighted with high precision scale and then placed in opaque test chambers (1L) covered with an air-tight glass lid. Chambers were installed in a temperature-controlled environment (set at 20°C, a temperature that does not elicit significant locomotor activity) 2 hours before beginning of trial. Outside air was drawn into each test chamber using a Bioblock Scientific 55 L air pump for 5 min ensuring +99% air turnover. Baseline samples of outside air were collected at the onset of each trial. Trials were performed during the night (12h) so that lizards were inactive most of the time. At the end of each trial, an air sample was collected from inside the chambers using two 150 ml syringes and we noted if the lizard defecated during the night (N=41). The baseline and final CO<sub>2</sub> and O<sub>2</sub> concentrations were determined using high precision gas analyzers (CA10 & FC10A, Sable Systems, Las Vegas, NV). Air was pushed from the syringes using an infusion pump (KDS210; KD Scientific, Inc., Holliston, MA, USA), passed through Drierite to remove water and then sent at a controlled rate to the analyzer calibrated before each trial. With baseline and final concentrations, we calculated the VO<sub>2</sub> (volume of O<sub>2</sub> consumed in ml per hour) and the respiratory quotient as the ratio of CO<sub>2</sub> produced by O<sub>2</sub> consumed. The respiratory quotient varied around 0.75 (mean =  $0.77 \pm 0.032$ , range = 0.695-0.911), which corresponds to the situation of a fasting animal that catabolizes lipids (Schmidt-Nielsen, 1997). We therefore subsequently analyzed resting metabolic rate using VO<sub>2</sub> values.

At Day 7, fast was ended and at Day 8 we used an evaporimeter (Aquaflux BIOX AF200, Biox Systems Ltd, UK) that quantified the trans-epidermal water vapor flux from measurements of a humidity gradient within a closed chamber (Imhof et al., 2009; Guillon et al., 2013). We measured independently cutaneous evaporative water loss (CEWL rate, g of water per m<sup>2</sup> per hour) from 4 locations on the back of each lizard in a laboratory room maintained at 23°C. We used an in vivo nail cap with rubber O-ring (diameter 2.6 mm) to ensure a complete seal between the measurement chamber and the lizard's skin. The evaporimeter was calibrated at the beginning of each trial following manufacturer procedures. We then gently pressed the

probe against the skin of the lizard for a few minutes to perform measurements. Each measurement trial ended once real time CEWL reading, monitored on a graphic interface, was stabilized ( $\pm 0.01 \text{ g.m}^{-2}.\text{h}^{-1}$  for 10 s). If any movement caused a leak in the seal between the measurement chamber and the animal's skin, we repeated the trial. In total, we performed 4 measurements for each animal and evaluated a posteriori their quality according to the shape of the curve as recommended by the manufacturer. We kept only good quality measurements for subsequent analyzes (range = 2-4 per lizard, mean = 3.66). Repeatability for measurements on the same individual was estimated using the linear mixed-effects model method and was significant ( $R = 0.56 \pm 0.04$ ,  $p < 0.0001$ ).

## 2.5. Statistical analyses

All statistical analyses were performed in R software version 3.5.3 (2019-03-11) "Great Truth" (RC Team 2019). In our results, we provide the standard deviation of the mean as the dispersion measure (mean  $\pm$  SD). In a first set of analyses, we compared measurements obtained in the two clades of common lizards and the wall lizards (*Z. vivipara louislantzi*, *Z. vivipara vivipara* and *P. muralis*) with an analysis of variance. For each measurement, we performed an ANCOVA test controlling for inter-individual differences in body mass. In a second set of analyses, we studied geographic variation among oviparous populations of *Z. vivipara louislantzi*. To test for potential correlations between morphological, behavioral and physiological traits on one hand and the population characteristics on the other hand, we used a two-step model selection procedure. We analyzed independently variation of body size (SVL, mm), body mass (W, g), body surface (mm<sup>2</sup>), thermal preferences ( $T_{\text{pref}}$ , °C), total water loss (TEWL, mg per hour), cutaneous water loss (CEWL,  $\text{g.m}^{-2}.\text{h}^{-1}$ ) and resting metabolic rate ( $\text{VO}_2$ , ml per hour) using linear mixed models with the function lme from the package nlme (Pinheiro et al., 2006). For each variable, we followed the same model selection procedure (as shown in Supplementary Tables S3 to S11).

Given that our variables of interest were influenced by both individual-level population-level factors, we proceeded in two steps. First, we fitted a full model including all individual and experimental covariates as fixed effects and then selected a first minimum adequate model using backward model selection based on AIC. We also assessed homoscedasticity and normality of residuals using the full model. Experimental covariates included measurement conditions (time of the day, temperature and humidity in the room when the experiment was not performed in climatic chambers), and presence of feces for TEWL and  $\text{VO}_2$ . Individual

covariates included age class, SVL, body surface and/or body mass depending on traits (see details below and in Supplementary tables S3-S11). Full models included additive linear effects of all covariates. For thermal preferences data, we analyzed all body temperatures recorded for each individual rather than a composite variable (e.g., mean or variance). Population identity was always included as a random effect to control for non-independence among individuals from the same population. Individual identity nested in the population identity was also included as a random effect to account for repeated measurements on the same individual for  $T_{\text{pref}}$  and CEWL data. In a second step, we tested if traits differed significantly among populations due to differences in climate or habitat. We used a model averaging procedure using the Akaike information-based criterion (AIC) comparison procedure (Burnham & Anderson, 2004) to compare the relative importance (sum of AICc corrected for small sample size over all models in which the variable appears) of environmental variables related to elevation, climate conditions (mean temperature and rainfall during activity season, pluviometry quotient), and habitat features (water presence and forest cover). The model averaging procedure was performed with maximum likelihood estimates of model parameters using the MuMIn package (Burnham & Anderson, 2002; Barton & Barton, 2015). Models were constructed from the best model selected during the first step described above in which we added the environmental variables alone as a fixed effect or the additive effects of one variable describing climatic conditions and one variable describing habitat features. Correlations between the environmental variables and the habitat variables were low since we chose to sample populations with as much contrasted access to water and forest cover along the altitude and aridity gradients as possible. For the purpose of model averaging calculations, all continuous covariates were centered and scaled and categorical covariates (age, water presence and forest cover) had their contrasts summed to zero. We selected the main effects with significant contributions to the variability of the traits based on the AICc difference between models, the relative weight of the models and the importance as well as conditional averages from model averaging procedure (Burnham & Anderson, 2004). If the minimal model was included in the set of best models, this implies uncertainty in the importance of environmental variables. If numerous concurrent best models were observed, this implies uncertainty about which environmental variable explains the most geographic variation in the trait. All model averaging tables and conditional averages of the important environmental variables are reported in the supplementary materials (Tables S3 to S11).

To complement these independent analyses of each trait, we further performed a path analysis with Piecewise Structural Equation modeling using the R package PiecewiseSEM (Lefcheck, 2016). This procedure allowed us to test causal paths identified in our model selection approach in a single model accounting for the multivariate relationships between our interrelated traits (see Supplementary information S13 for more details).

### 3. Results

#### 3.1. Differences between taxa

We found significant differences for all thermo-hydroregulation traits between the wall lizards and the two groups of common lizards, but not between oviparous and viviparous populations of the common lizard, even after controlling for allometric relationship (Table 1, see details in Supplementary Table S2). TEWL was 40% lower in *P. muralis* than in the two clades of *Z. vivipara* after controlling for body mass (*P. muralis*: TEWL = 0.853 mg.g<sup>-1</sup>.h<sup>-1</sup>; *Z. vivipara louislantzi*: TEWL = 1.387 mg. g<sup>-1</sup>.h<sup>-1</sup>; *Z. vivipara vivipara*: TEWL = 1.489 mg. g<sup>-1</sup>.h<sup>-1</sup>; ANCOVA: Taxa: F<sub>2,140</sub> = 10.9, p < 0.0001; Body mass: F<sub>1,140</sub> = 77.2, p < 0.0001). Similarly, CEWL was 40% smaller in *P. muralis* than in the two clades of *Z. vivipara* (ANOVA: Taxa: F<sub>2,652</sub> = 66.3, p < 0.0001). VO<sub>2</sub> rates corrected for body mass, were 26% lower in *P. muralis* than in *Z. vivipara* (ANCOVA: Body mass: F<sub>1,160</sub> = 336.6, p < 0.0001, Taxa: F<sub>2,160</sub> = 9.63, p = 0.0001). *T*<sub>pref</sub> were significantly lower in *P. muralis* compared to *Z. vivipara* subspecies (ANOVA: F<sub>2,1640</sub> = 47.19, p < 0.001). None of the post-hoc tests revealed any difference between *Z. vivipara louislantzi* and *Z. vivipara vivipara* (**Table 1**).

**Table 1.** Mean body mass, water loss rates, oxygen consumption rates and thermal preferences in the two sub-species of *Z. vivipara* and in *P. muralis*. Values are the mean values for respectively 142 *Z. vivipara louislantzi*, 21 *Z. vivipara vivipara* and 17 *P. muralis* adult males (± standard deviation).

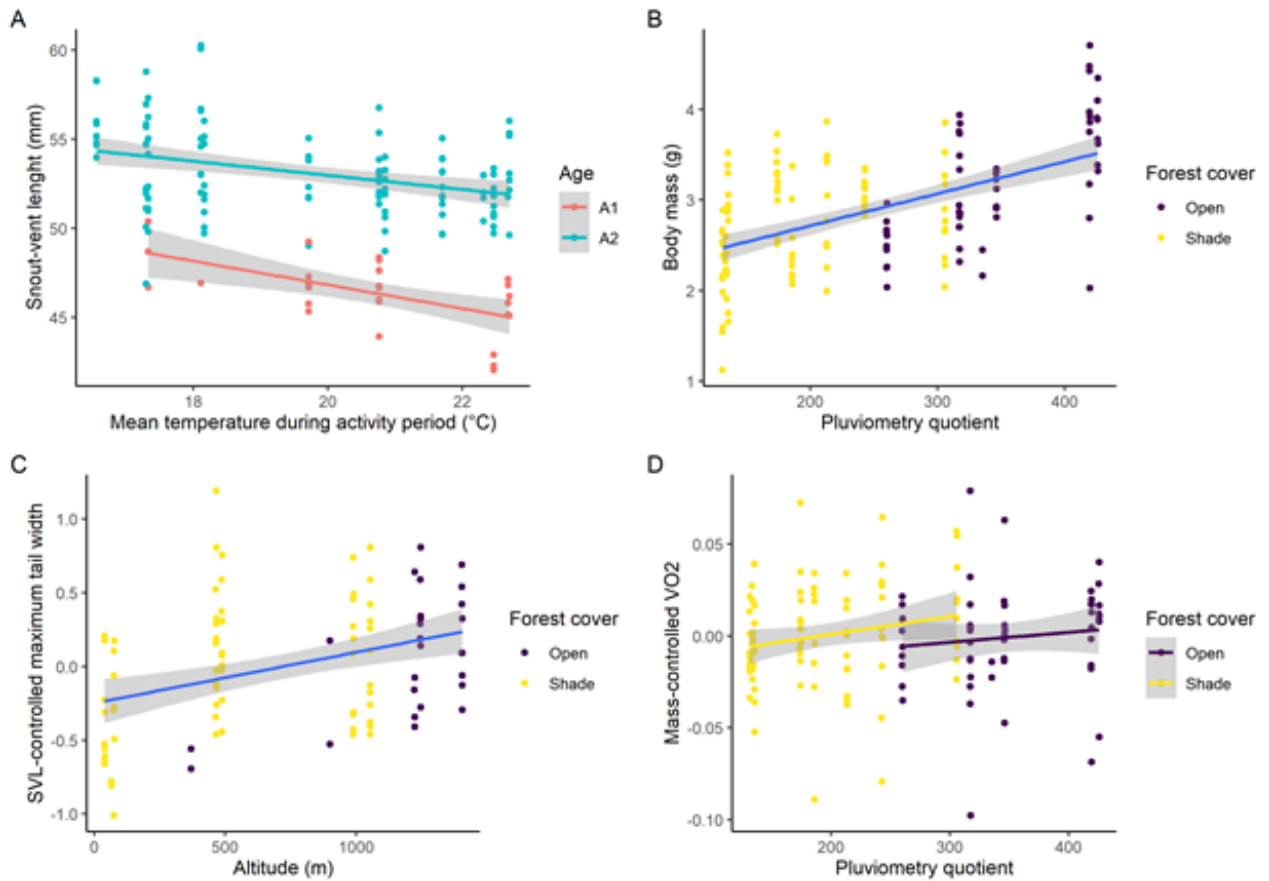
|                                   | <i>Z. vivipara louislantzi</i> | <i>Z. vivipara vivipara</i> | <i>P. muralis</i> |
|-----------------------------------|--------------------------------|-----------------------------|-------------------|
| BM (g)                            | 2.90 ± 0.66                    | 3.15 ± 0.75                 | 5.26 ± 1.60       |
| TEWL (mg/h)                       | 3.63 ± 0.78                    | 3.97 ± 0.68                 | 4.08 ± 1.70       |
| TEWL per mass (mg/h/g)            | 1.42 ± 0.29                    | 1.54 ± 0.27                 | 0.87 ± 0.24       |
| CEWL (g/m <sup>2</sup> /h)        | 16.92 ± 4.35                   | 17.86 ± 3.11                | 10.59 ± 2.23      |
| VO <sub>2</sub> (ml/h)            | 0.186 ± 0.03                   | 0.203 ± 0.03                | 0.254 ± 0.06      |
| VO <sub>2</sub> per mass (ml/h/g) | 0.075 ± 0.013                  | 0.073 ± 0.012               | 0.058 ± 0.008     |
| <i>T</i> <sub>pref</sub> (°C)     | 34.94 ± 2.44                   | 34.67 ± 2.75                | 32.08 ± 3.51      |

### 3.2. Morphological variation among oviparous populations of *Z. vivipara*

The SVL and body condition (body mass corrected for SVL with a linear regression) varied among populations (ANOVA: Population effect:  $F_{14,127} = 4.7$ ,  $p < 0.0001$  and  $R_{\text{interpop}} = 0.38$  for SVL and ANOVA: Population effect:  $F_{14,126} = 7.0$ ,  $p < 0.0001$  and  $R_{\text{interpop}} = 0.42$  for BM) and these differences were partly explained by climatic conditions. The most important environmental and individual variables explaining variation of SVL included the age class (contrast between age 1 and older males =  $-3.2 \pm 0.25$  mm,  $p < 0.0001$ ), mean temperature during activity period (slope =  $-0.5 \pm 0.16$  mm per °C,  $p = 0.01$ ) and the pluviometry quotient (slope =  $0.01 \pm 0.005$  mm per Q unit,  $p = 0.04$ , see Table S3). Thus, lizards had a larger average body size in colder climates (Figure 2A). The most important environmental variables explaining geographic variation of body condition were the pluviometry quotient (slope =  $0.002 \pm 0.001$  g per Q unit,  $p = 0.03$ ), the altitude (estimate =  $0.0003 \pm 0.0001$  g per m,  $p = 0.03$ ) and the mean temperature during activity period (estimate =  $-0.07 \pm 0.03$  g per °C,  $p = 0.04$ ). According to the best supported model, lizards had a higher body condition in highland populations with a high pluviometry quotient and in open habitats than under dense forest cover (see variations of body mass in Figure 2B, Table S4). Body surface was as expected strongly correlated with body mass ( $r = 0.9$ ,  $p < 0.0001$ ) and followed a similar pattern of geographic variation than body condition (see Table S5).

Tail width at the three recorded positions was correlated with SVL and body mass and the different measurements of tail width were strongly correlated with each other (all  $r > 0.75$ ,  $p < 0.0001$ ). We present only the analyses on maximum tail width and tail width at 10th subcaudal scale. For both, the most important environmental and individual variables included the SVL (for max width : slope =  $0.046 \pm 0.021$  mm,  $p = 0.03$  ; for 10th scale : slope =  $0.074 \pm 0.015$  mm per mm,  $p < 0.0001$ ), the altitude (for max width : slope =  $0.0006 \pm 0.0002$  mm per m,  $p = 0.0009$  ; for 10th scale : slope =  $0.0005 \pm 0.0001$  mm per m,  $p = 0.0003$ , see Figure 2C, Tables S6), the mean temperature during activity period (for max width : slope =  $-0.11 \pm 0.04$  mm per °C,  $p = 0.018$  ; for 10th scale : slope =  $-0.09 \pm 0.03$  mm per °C,  $p = 0.006$ ) and the mean precipitation during activity period (for max width : slope =  $0.001 \pm 0.0007$  mm per mm,  $p = 0.046$  ; for 10th scale : slope =  $0.001 \pm 0.0005$  mm per mm,  $p = 0.03$ ). The best supported models retained the effect of age class and SVL as individual covariates and of altitude and forest cover, indicating a joint effect of climate conditions and local habitat (see Figure 2C, Tables S6).

**Figure 2:** Spatial variation of morphological and physiological traits in an environmental gradient. **A:** Snout to vent length variation along a gradient of mean environmental temperature during activity period; color code represents the age class of the lizards (A1=1-year-old, A2=over-1-year-old). **B:** Body mass variation along a gradient of aridity of the environment (low values indicate less mesic environments); color separates forest covered habitats (yellow) from open habitats (purple). **C:** Maximum tail width (mm) relative to SVL (residuals of a linear regression) along a gradient of altitude; color separates forest covered habitats (yellow) from open habitats (purple). N=94 due to missing values. **D:** Volume of oxygen consumed (ml per hour) relative to the body mass (residuals of a linear regression) according to the aridity of the environment; color separates forest covered environments (yellow) from open environments (purple).



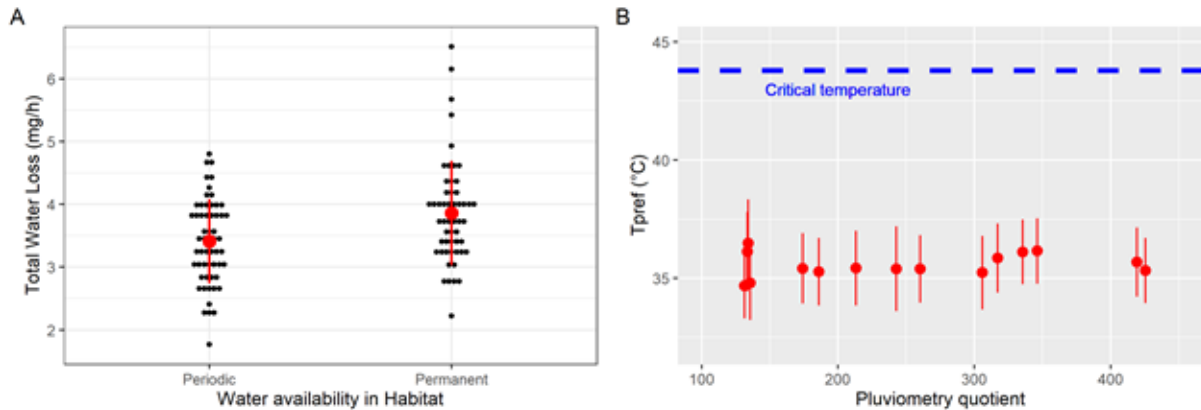
### 3.3. Physiological variation among oviparous populations of *Z. vivipara*

The TEWL at rest increased with body mass (estimate =  $0.0008 \pm 0.0001$  mg/h per g,  $p < 0.0001$ ). There was some degree of variation in TEWL among populations (ANOVA: Population effect:  $F_{14,94} = 3.5$ ,  $p = 0.0001$  and  $R_{\text{interpop}} = 0.37$ ) and our model comparison procedure uncovered that the permanent access to free water in the habitat was a significant variable explaining this geographic variation (Figure 3A, Table S7). Lizards living in habitats with no permanent access to water had lower TEWL (contrast =  $-0.26 \pm 0.07$ ,  $p = 0.001$ ). The effects of other variables were more uncertain with models suggesting that TEWL decreased weakly with altitude and in open habitats compared to forest habitats (Table S7). The CEWL values showed a much greater variability among individuals within a population than among populations ( $R_{\text{interpop}} = 0.22$ ;  $R_{\text{interind}} = 0.47$ ). The model that best explained CEWL variation included only experimental conditions effects with a positive correlation of the absolute humidity of the room at the time of measurement (estimator =  $0.70 \pm 0.04$ ,  $p = 0.0001$ , Table S8).

Apart from the significant positive effect of body mass on  $\text{VO}_2$  at rest (estimate =  $6.14 \cdot 10^{-5} \pm 5.10 \cdot 10^{-6}$  ml/h per mg,  $p < 0.0001$ ), there was some uncertainty about the effects of environmental variables on  $\text{VO}_2$  despite variation among populations (ANOVA: Population effect:  $F_{17,144} = 1.91$ ,  $p = 0.02$  and  $R_{\text{interpop}} = 0.25$ ). According to the best supported model, lizards tended to have higher  $\text{VO}_2$  in populations with a high pluviometry quotient, and, in addition, in forest habitats than in open areas (Figure 2D, Table S9).

Mean plasma osmolality was  $306 \pm 18$  mOsm  $\text{kg}^{-1}$  and was different among populations (ANOVA: Population effect:  $F_{14,119} = 3.8$ ,  $p < 0.0001$  and  $R_{\text{interpop}} = 0.35$ ), but we found no significant effect of environmental variables on plasma osmolality (Table S10).

**Figure 3:** Variation in oviparous populations of *Z. vivipara* A. Rates of total evaporative water loss (TEWL) according to the availability of water in the habitat; red dot is mean value and red lines are standard deviations. B. Mean and standard deviation of preferred body temperatures ( $T_{\text{pref}}$ ) across populations along a gradient of aridity of the environment (low values indicate less mesic environments). The blue dashed line is the critical thermal maximum of *Z. vivipara* (Gvoždík & Castilla, 2001).



### 3.4. Variation of thermal preferences in oviparous populations of *Z. vivipara*

Thermal body preferences were relatively high in our sample (mean = 34.94°C, range = 19.80-39.40°C, Figure 3B) compared to previous studies with the same species (e.g.,  $T_{\text{pref}} = 31.5$ ; Gvoždík & Castilla, 2001; Trochet et al., 2018).  $T_{\text{pref}}$  showed a great intra-population variability but varied little across populations (ANOVA: Population effect:  $F_{14,1420} = 5.11$ ,  $p < 0.001$  and  $R_{\text{interpop}} = 0.18$ , see Figure 3B) and this geographic variation was not significantly explained by environmental variables. Our model comparison procedure only revealed the effect of experimental covariates and suggested some potential but weak effects of forest cover (not significant in conditional averages, Table S11). Lizards living in open habitats tended to have higher  $T_{\text{pref}}$  than lizards from forest habitats.

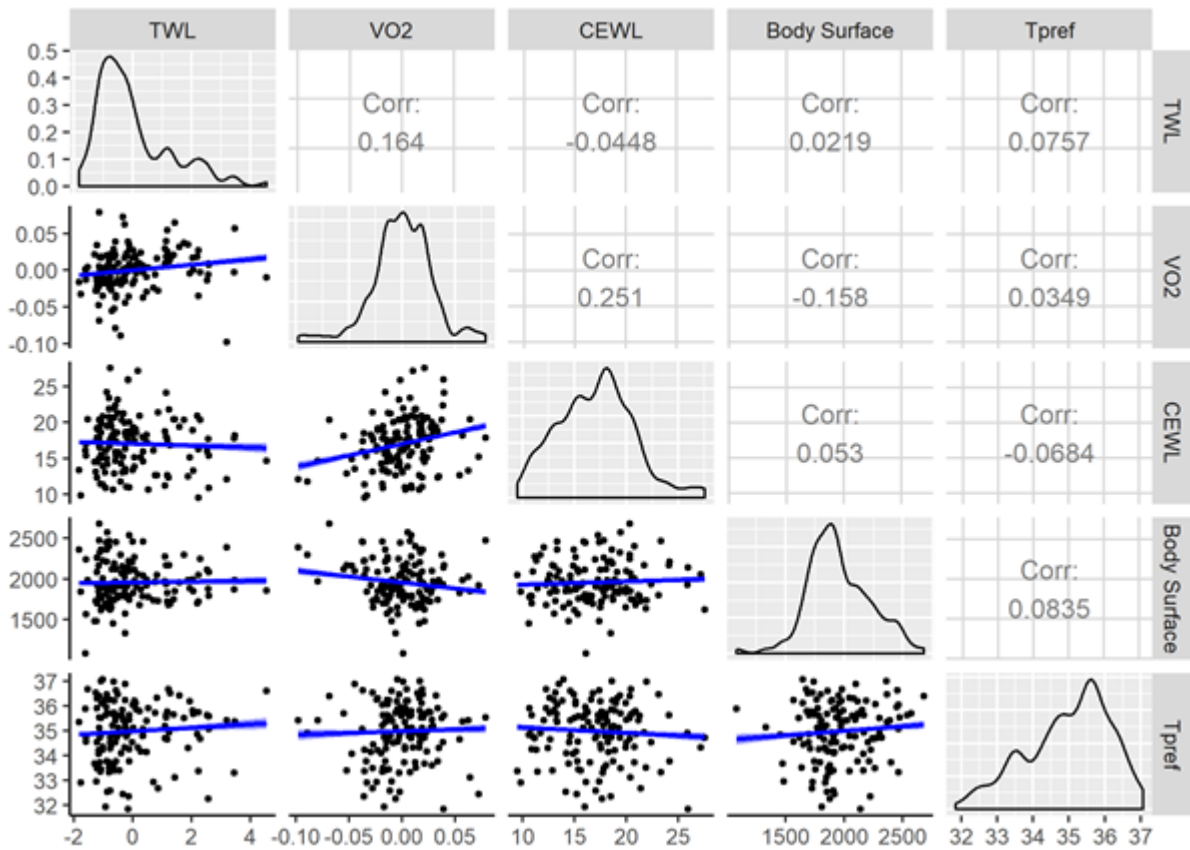
### 3.5. Covariation between thermo-hydroregulation traits

We found no significant correlation between TEWL, CEWL,  $\text{VO}_2$ , the body surface area of the animal and the  $T_{\text{pref}}$  (Figure 4). First, TEWL,  $T_{\text{pref}}$  and  $\text{VO}_2$ , which provide three potentially related information about the thermo-hydroregulation strategy of lizards, were not strongly correlated (Pearson product moment correlation, all  $r < 0.3$ , all  $p < 0.05$ ). Second, we explored the relationship between TEWL and the potential subcomponents of evaporative water losses, such as CEWL, body surface and ventilation rate (which is linearly related to  $\text{VO}_2$ ), but we found no correlation between them (all  $r < 0.2$ , all  $p > 0.05$ ). In addition, in the SEM result CEWL and  $\text{VO}_2$  were weakly positively correlated ( $r = 0.26$ ,  $p = 0.018$ , see Supp. Info Appendix



S1). Otherwise, model comparison with AIC gives similar results to those of the piecewiseSEM procedure (see Appendix S1).

**Figure 4:** Scatterplot matrix of total and cutaneous water losses (TEWL,  $\text{mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$  and CEWL,  $\text{g}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ), metabolic rate ( $\text{VO}_2$ ,  $\text{ml}\cdot\text{h}^{-1}$ ), total body surface ( $\text{mm}^2$ ) and preferred body temperature ( $T_{\text{pref}}$ ,  $^{\circ}\text{C}$ ). The diagonal represents the distribution of each variable, the upper triangle provides the Pearson correlation coefficients between pairs of variables, and the lower triangle provides scatter plots of each pair of variables with the best linear regression line.



#### 4. Discussion

Studies of intraspecific variation in functional traits across environmental gradients have tended to focus on the thermal biology of ectotherms instead of the joint variation and integration of their thermo-hydroregulation strategies (Artacho et al., 2017; Domínguez-Guerrero et al., 2021). Here, we compared ecophysiological traits linked to both water balance and body temperature regulation in populations of *Zootoca vivipara louislantzi* ranging from rear edge nearby sea level to the leading edge of their distribution in highland habitats. In addition, we compared these populations with two outgroups including a different species and another subspecies, with which we expected substantial ecophysiological differences.

Strong ecophysiological variation was indeed observed between wall lizards *Podarcis muralis* and common lizards *Zootoca vivipara*, as expected given the broad ecological and habitat differences between these two species. The wall lizard is found on average in warmer and drier areas than the common lizard and this species also exhibits distinct preferences for dry saxicolous rather vegetated habitats where common lizards thrive, even though these two species can occur sympatrically (Mole, 2010). On the one hand, *Podarcis muralis* had lower water loss rates (both TEWL and CEWL) and basal metabolic rates ( $VO_2$ ) than *Zootoca vivipara*. Comparative studies of squamate reptiles and other vertebrates have uncovered similar findings, with higher resistance to evaporative water loss and lower basal energy expenditure in species from hot and dry environments (Dupoué et al. 2017, Le Galliard et al., 2021a, Cox & Cox, 2015). On the other hand, *P. muralis* displayed lower preferred temperatures ( $T_{pref}$ ) than our populations of *Z. vivipara* instead of the predicted higher  $T_{pref}$  expected for organisms from warmer habitats (Garcia-Porta et al., 2019). Previous studies of *P. muralis* and *Z. vivipara* have found that the  $T_{pref}$  of adult males can vary seasonally with high  $T_{pref}$  during the early mating season, lower  $T_{pref}$  during the spring season and then higher  $T_{pref}$  during the summer season (Osojnik et al., 2013). Thus, one possibility is that wall lizards, who tend to have an earlier phenology than common lizards, had lower  $T_{pref}$  because they were sampled slightly later during their mating season than common lizards. Another possibility is that the relatively smaller  $T_{pref}$  of wall lizards reflects adjustments to water limitation and habitat aridity in their native population, as shown in laboratory experiments (Sannolo & Carretero, 2019; Le Galliard et al., 2021b).

We found no significant ecophysiological variation between the two clades of *Zootoca vivipara* despite their ancient evolutionary divergence and a major difference in reproduction mode between the two clades. Phylogenetic studies revealed six major genetic clades for *Zootoca vivipara* in Eurasia with four widespread viviparous clades present in most of the distribution range and two oviparous clades restricted to the southern margin of the range distribution in Western or Eastern Europe (Surget-Groba et al., 2006). The most parsimonious evolutionary scenario proposes that viviparity evolved only once but then a reversal back to oviparity occurred in the Western populations from our study area (Recknagel et al., 2018; Horreo et al., 2018). This transition back to oviparity is associated with climate warming, since oviparous forms are generally favored over viviparous forms in warmer habitats (Shine, 1985). The two reproductive modes are probably separated given the limited gene flow and reproductive isolation between them (e.g., Cornetti et al., 2015). Viviparity is considered as a key

evolutionary transition to life under cold climatic conditions (Horreo, Jiménez-Valverde, & Fitze, 2021), with oviparous lizards being adapted to warmer and more variable environment during the reproductive season (as suggested by Horreo et al., 2021). This scenario led us to expect major differences in thermo-hydroregulation strategies between clades B (oviparous) and E (viviparous). However, we found no physiological or behavioral adaptation as the two clades were very similar in terms of TEWL,  $T_{pref}$  and  $VO_2$ . In our experiment, interindividual and interpopulation differences on these traits were stronger than the divergence due to clades. However, we encourage further studies of the ecophysiological strategies of adult females, especially during gestation when phenotypic differences between the two reproductive modes are likely to be stronger (Recknagel & Elmer, 2019).

Morphological differences among oviparous populations were explained by climatic conditions including temperature and the pluviometry quotient  $Q$ . First, body condition of males was explained by both environmental temperature and humidity: sexually mature males were on average heavier in the more mesic habitats (*sensu*  $Q$  index), whereas relative tail width increased with altitude (and cold temperatures) and with rainfall. In this species, caudal width represents a good proxy of tail reserves, which are an important body component for fat and protein storage whose variation can reflect changes in food availability, energy intake and also water availability (Bateman & Fleming, 2009; Bruschi et al., 2020). Body condition might increase with humidity because of positive effects of rainfall and water availability on habitat quality, including food availability, but this should be accompanied by an increase of body size with humidity. Alternatively, low environmental temperatures and high water availability might reduce energy expenditure and the reliance of lizards on fat or protein catabolism to restore energy and water balance (e.g., Bruschi et al., 2020). Hence, we can hypothesize that males from rear edge, drier and hotter populations were more prompt to catabolize tail reserves, probably to fuel some higher metabolic demand (in warmer climates) and to support water demands (in drier climates). Further examinations of physiological traits in standard conditions tend to confirm the later hypothesis.

Second, size was influenced mostly by environmental temperature: sexually mature males were on average longer in the colder habitats. This geographic variability for body size is globally consistent with previous data collected across the altitudinal range of other ectothermic vertebrates (Peterman, Crawford, & Hocking, 2016; Trochet et al., 2019). These two aspects suggest a positive effect of cold temperature and humidity on body size and body conditions. If the effect on size has been documented previously (Lu et al., 2018; Roitberg et al., 2020), the

effect of aridity is rarely tested. A recent thorough analysis of the body size variation across the range distribution of *Z. vivipara* suggested that body size varies non-monotonously with the length of the activity season in this species because of the underlying thermal plasticity of growth and sexual maturation (Roitberg et al., 2020). This scenario of thermal plasticity predicts a shift towards a larger mean body size of adult individuals in colder climates because age at first reproduction is delayed by a year or more in a seasonal environment (Adolph and Porter, 1996). Thus, adult males are bigger in colder climates because they mature older and therefore at a larger body size on average than adults growing at higher temperatures but maturing earlier in life (Atkinson, 1994; Angilletta, 2009). However, under this scenario, we would not expect the size of young males of the year (age class A1) to be smaller in warmer habitats if those yearlings are born earlier and grow faster than those of colder habitats (Sorci et al., 1996; Roitberg et al., 2020), which is what we found here. Our sample of 1-year-old individuals was however likely biased as we only captured sexually mature young males, whereas most young are not mature at this age, especially in cold climates. We therefore performed a complementary analysis of a larger dataset including many more *Z. vivipara* populations and both sexually mature and immature young males of the year. In this supplementary database, we had a much stronger variability in the body size of young males of the year. This updated analysis confirmed a positive thermal cline for the body size of 1 year-old males with SVL increasing by  $1.03 \pm 0.29$  mm per °C mean activity temperature ( $p = 0.0006$ , see supplementary Table S12). This reverse thermal clines for body size in young males of the year and older males conforms exactly with the “saw-tooth” relationship between body size and seasonality proposed by Adolph and Porter (1996) and seen across the broad geographic distribution of *Z. vivipara* across Europe (Roitberg et al., 2020). Altogether, these results indicate that broad scale climate gradients instead of local habitat features were the main determinants of geographic variation in morphology.

Lizards living in habitats with temporary access to water had lower total evaporative water loss rates than those from habitats with permanent access and the plasma osmolality was remarkably consistent across habitat types and climate conditions, similar to a previous finding in adult males and females of the viviparous clade E in Massif Central, France (Dupoué et al., 2017b). This confirms that common lizards can adjust or adapt their total water loss to cope with seasonal habitat dryness and that water availability in the environment is more consistently related to this functional trait of the water budget than rainfall or thermal conditions. We also examined traits involved in TEWL variation including body surface (which relates to the total

exchange area for cutaneous evaporative water loss), standard cutaneous water loss rates (which relates to skin permeability to water loss), and  $VO_2$  at rest (which relates to basal energy expenditure and also correlates with respiratory water loss). Quite surprisingly, we found no effect of water availability in the habitat on these three traits and little inter-individual correlation between these traits and TEWL, which does not allow us to point a specific avenue by which lizards down-regulated standard TEWL rates in habitats with temporary access to water. One possibility is that some behavioral mechanisms were driving the observed pattern. For example, ocular water loss can represent a significant avenue for TEWL in small lizard species (Pirtle et al., 2019) and the time spent with eyes open could be variable during inactivity periods.

Contrary to our expectations, the  $VO_2$  of adult males were poorly influenced by climate conditions, especially environmental temperatures, except for a trend towards higher  $VO_2$  in more mesic conditions that would require further testing with a larger sample size. Note that this weak pattern of variation of  $VO_2$  with climate aridity is the same than the one seen in some desert species of birds and rodents (McNab & Morrison, 1963; Tieleman, Williams, & Bloomer, 2003). We also found a small positive effect of forest cover on  $VO_2$  but this effect remains difficult to interpret given that closed habitats tended to be more frequent at a lower elevation. Given the strong and consistent differences in thermal and rainfall conditions between the relict lowland populations and those in highland mountains, we expected that the  $VO_2$  of adult males would be significantly lower in low altitude populations (i.e. metabolic cold adaptation or Krogh's rule described in Krogh & Lindhard, 1914) as it has been shown in other species of lizards (e.g. Plasman et al., 2020). However, a recent review by Pettersen (2020) on reptiles demonstrates little support for counter gradient variation in metabolic rate (which would reflect an adaptive response to geographic gradient), whereas it did reveal such an adaptive response on development time. Here, we do not find a difference in  $VO_2$  at adult stage across a 2000 m elevation gradient but cannot exclude that geographic variation may be more substantial at earlier stages of life or in females, but also at a different season, for example at the end of the summer following seasonal acclimatization of resting metabolic rate.

Regarding thermoregulation behavior, we studied thermal preferences to quantify thermoregulation strategies under controlled laboratory conditions. Variation in  $T_{pref}$  was often higher among individuals within a population than among populations and fell 6-7°C below the critical thermal maximum (Gvoždík & Castilla, 2001; Trochet et al., 2018). Individual differences in  $T_{pref}$  were independent from variation in water loss rates or basal metabolism.

The  $T_{\text{pref}}$  also remained similar on average among populations with contrasted climatic and habitat conditions confirming the results of several previous comparative studies of thermal preferences in this species in other geographic areas (Van Damme, Bauwens, & Verheyen, 1990; Gvoždík & Castilla, 2001; Rozen-Rechels et al., 2021; Carretero, Roig, & Llorente, 2005). Note that we cannot exclude stronger geographic differences of  $T_{\text{pref}}$  later in the season due to different acclimatization conditions across our geographic gradients, as a strong seasonality effect was found in a meta-analysis on thermoregulation efficiency of lacertid lizards (Ortega & Martín-Vallejo, 2019). On the same species, Trochet et al. (2018) found a slight decrease of the  $T_{\text{pref}}$  of adult males and females from ca. 31-32°C at 500 m elevation to ca. 29-30°C at 2000 m elevation during the late spring season. Irrespective of this possibility, the fact that  $T_{\text{pref}}$  varied little across a temperature gradient of 6°C for mean temperature during the activity season suggests that behavioral thermoregulation is optimized at grossly similar body temperatures over the geographic gradient, perhaps because the species is a thermal generalist (Angilletta et al. 2002).

Assuming a strong selection for optimization of thermal performances, behavioral adjustments may explain the absence of geographic variability in  $T_{\text{pref}}$ . We know that lizards can use microhabitats (e.g., rocks, shaded vegetation or burrows) to maintain an optimal body temperature and buffer the negative effects of cold or hot environmental temperatures on their performance (Gaudenti et al., 2021; Taylor et al., 2021). Hence, macroclimatic conditions, here quantified by average air temperatures, might be less relevant than microclimatic conditions since common lizards can shift microhabitat selection to keep an optimal temperature even in the extreme parts of our geographic gradient (Rozen-Rechels et al., 2021). This supposes a great spatial variation in operative temperature to allow efficient thermoregulation even in cold and warm environments (Logan, van Berkel, & Clusella-Trullas, 2019). Ultimately, some components of the ecological niche of the species could also change along the climatic gradients. For example, there might be a shift toward sunny, low vegetation habitats in high altitude populations compared to more shaded forest habitats in lowland populations. Complementary studies of the spatial distribution of contemporary populations are required to confirm this hypothesis. However, our thermal cline was quite substantial and we found strong morphological differences along the gradient, which were likely caused by differences in the length of the activity season. This suggests that body temperature regulation was to some extent constrained by cold climate conditions early and late in the activity season. Another compensatory mechanism could be the possibility of geographic changes in basking behavior

and effort (Stevenson, 1985): here, animals from cold populations could spend more time basking to keep an optimal temperature even at the cost of spending less time foraging (Buckley, Ehrenberger, & Angilletta, 2015). Yet, a previous study examined behavioral thermoregulation between lowland and highland populations of *Z. vivipara* and found no compensatory behavior of this kind in high altitude (Gvoždík, 2002). In any case, our results suggest that some aspects of the thermal preferences or the ecological niche did compensate at least partly for the strong macroclimatic differences in environmental temperatures. The behavioral mechanisms allowing common lizards to maintain optimal body temperature and performance across gradients of environmental temperatures are important to study in the future since they can shield organisms from environmental variation and cause evolutionary inertia of physiological traits (i.e., Bogert effect, Muñoz, 2021).

Overall, our study of intraspecific variation of morphology and thermo-hydroregulation traits in *Z. vivipara louisiantzi* indicate that macroclimate gradients were the main determinants of geographic variation in morphology but not for thermo-hydroregulation traits. Relict populations from the warm rear edge of the range distribution displayed no particular ecophysiological adaptations. Habitat features such as water access and forest cover had a stronger influence on EWL than thermal conditions. We suggest that habitat features should be included in future comparative studies of terrestrial ectotherms' physiology in order to better understand their role relative to climate conditions.

## **5. Acknowledgements and funding**

This study received the financial and technical support from the Centre National de la Recherche Scientifique (CNRS), and was funded by the Agence Nationale de la Recherche under the 'Aquatherm' project (ANR-17-CE02-0013 to JFLG), by the European Union (Feder), the regional council of Nouvelle Aquitaine and the departmental council of Gironde & Pyrénées-Atlantiques under the 'Climate Sentinels' project (coordinators: Cistude Nature, Fanny Mallard), and under the 'Aquastress' project (2018-1R20214 to OL). Permission to capture and handle lizards was provided by DREAL/21-2020 (GED: 12278) and the experiment was carried out in accordance with institutional guidelines, under license from the French Ministry of the Environment and the veterinary and environmental services of every region where we sampled lizards (permits APAFIS#19920-2019032323596391). We thank the two anonymous reviewers for their helpful comments to improve our manuscript.

## 6. Data Availability

The data underlying this article are available in Zenodo at <https://dx.doi.org/10.5281/zenodo.6366222>

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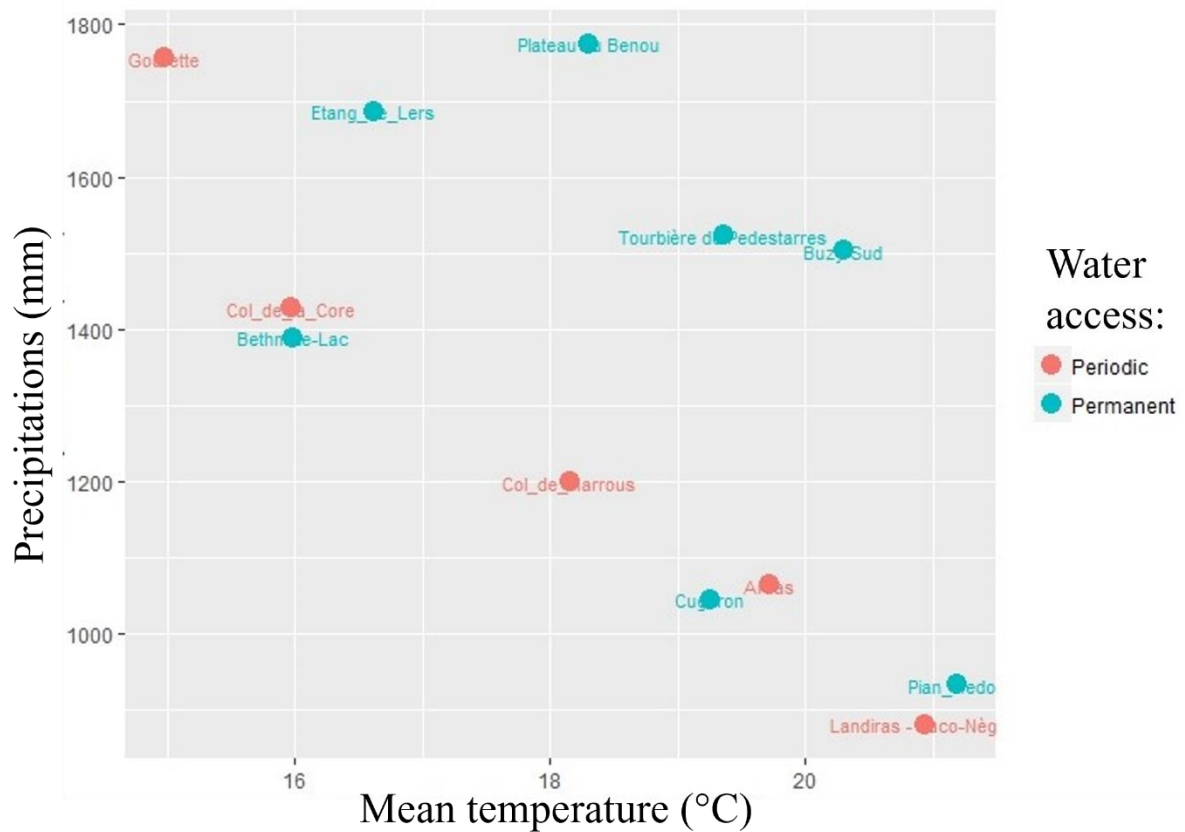
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## Supporting information

**Figure S1:** Measurement design for one lot (15 lizards)

| Before Day0 | Day0 to Day3      | Day4                                    | Day5                                      | Day6                                  | Day7        | Day8                                |
|-------------|-------------------|---|---|---------------------------------------|-------------|-------------------------------------|
| Acclimation | Beginning of fast | Measurement of total water loss at rest | Measurement of preferred body temperature | Measurement of resting metabolic rate | End of fast | Measurement of cutaneous water loss |

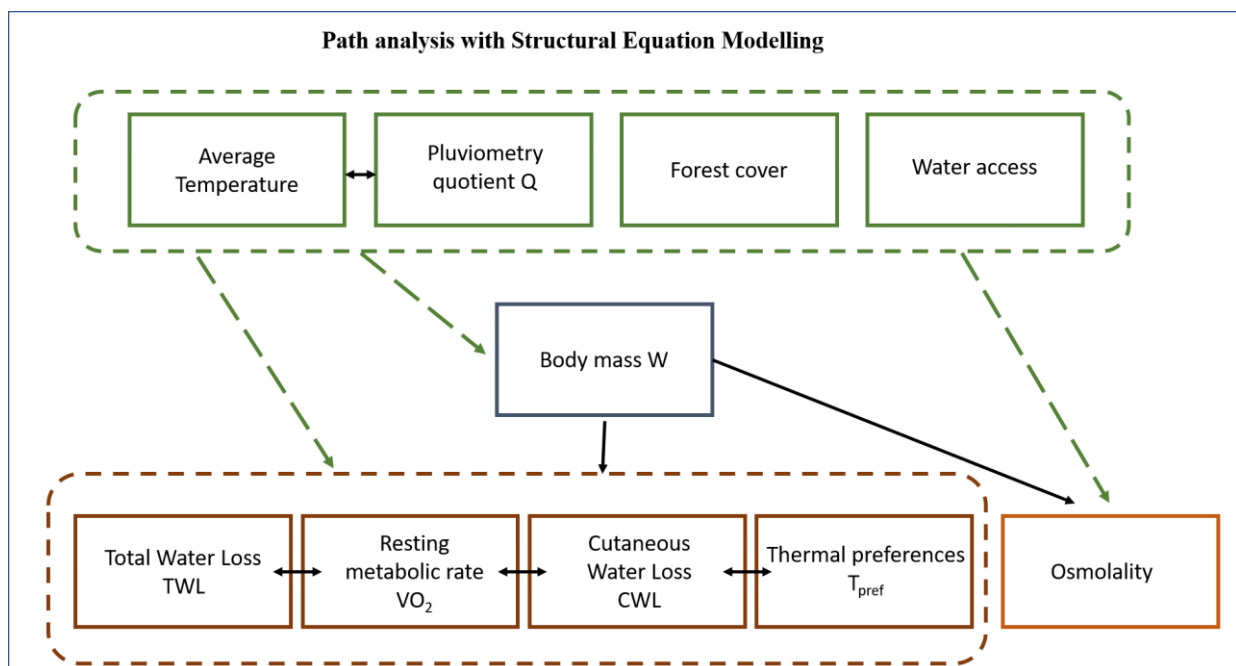
**Figure S2 :** Diversity of sampled populations in terms of climate and water access in the habitat





## Appendix S1: Complementary analysis of the multivariate data set using Structural Equation Modelling

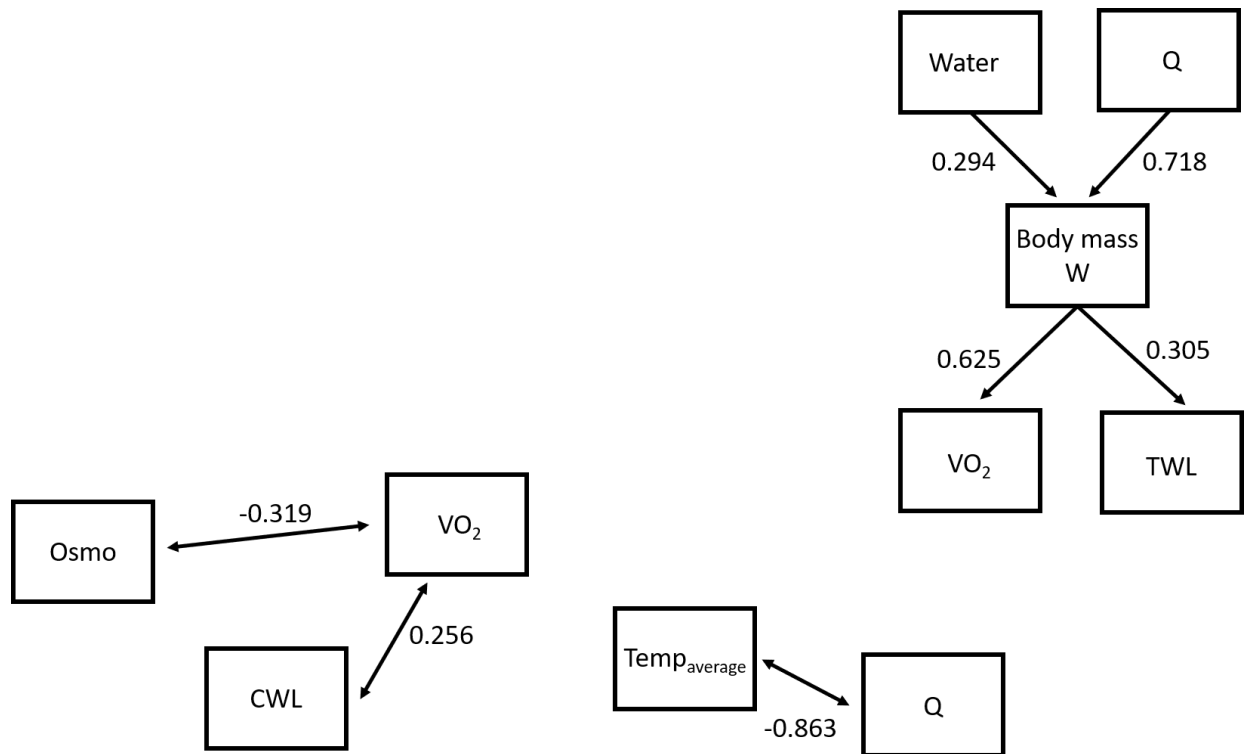
To further explore the relationships between ecophysiological traits and environmental conditions, we used piecewise structural equation modelling (SEM) with the *piecewiseSEM* v2.1 package in R (Lefcheck, 2016) in combination with linear mixed models using the R packages *nlme* (Pinheiro et al., 2019). We designed our full model based on the same philosophy than the univariate model selection approach described in the main text (see path diagram below). In our full model, average temperature, pluviometry quotient Q and the two habitat features (water access and forest cover) have direct, causal effects on body mass W. In addition, these 4 environmental variables and body mass have direct, causal effects on plasma osmolality (our index of physiological dehydration) on one side, and on each of the thermo-hydroregulation traits (TWL,  $VO_2$ , CWL and  $T_{pref}$ ) on the other side. Furthermore, we assumed correlated errors between average temperatures and pluviometry quotient and between the four ecophysiological traits. Based on an earlier analysis of this model with tests of direct separation (Lefcheck, 2016), osmolality and  $VO_2$  appeared to be correlated, so we added correlated errors between these two variables.



The summary of the outputs of this full model is presented below. The assessment of the goodness-of-fit showed that it fitted well the data (Fisher's C = 9.372 with P-value = 0.154). In this full model, we found that significant paths are the same than the ones uncovered in our other model selection approach in the main text. Indeed, except for body mass which is positively influenced by pluviometry quotient Q in addition to habitat features, the SEM showed that mostly habitat features and not thermal conditions influenced our ecophysiological traits (see coefficients of regression and critical test values below, significant causal paths are bolded).

| Model                               | Estimate | SE          | P-Value              | R <sup>2</sup> <sub>marginal</sub> | R <sup>2</sup> <sub>conditional</sub> |
|-------------------------------------|----------|-------------|----------------------|------------------------------------|---------------------------------------|
| W ~ Taverage                        | -0.0091  | 0.077 0.002 | 0.908                |                                    |                                       |
| Q                                   | 0.0049   | 0.25        | <b>0.038*</b>        | 0.33                               | 0.42                                  |
| Forest                              | 0.3467   | 0.148       | 0.196                |                                    |                                       |
| Water                               | -0.0466  |             | 0.759                |                                    |                                       |
| Osmo ~ W                            | -2.6415  | 3.045       | 0.387                |                                    |                                       |
| Taverage                            | 3.369    | 2.734       | 0.246                | 0.05                               | 0.21                                  |
| Q                                   | 0.0749   | 0.074       | 0.339                |                                    |                                       |
| Forest                              | 5.3381   | 8.957       | 0.564                |                                    |                                       |
| Water                               | -5.3288  | 5.238       | 0.333                |                                    |                                       |
| TWL ~ W                             | 0.6049   | 0.194       | <b>0.002**</b>       |                                    |                                       |
| Taverage                            | -0.2855  | 0.138       | 0.066                | 0.22                               | 0.28                                  |
| Q                                   | -0.0037  | 0.004       | 0.353                |                                    |                                       |
| Forest                              | 0.5216   | 0.456       | 0.279                |                                    |                                       |
| Water                               | 0.7528   | 0.271       | <b>0.019*</b>        |                                    |                                       |
| CWL ~ W                             | -0.5686  | 0.587       | 0.335                |                                    |                                       |
| Taverage                            | 0.8019   | 0.551       | 0.176                | 0.09                               | 0.26                                  |
| Q                                   | 0.03     | 0.015       | 0.073                |                                    |                                       |
| Forest                              | 1.6902   | 1.805       | 0.371                |                                    |                                       |
| Water                               | -0.7595  | 1.053       | 0.487                |                                    |                                       |
| VO <sub>2</sub> ~ W                 | 0.0462   | 0.005       | <b>&lt;0.0001***</b> |                                    |                                       |
| Taverage                            | -0.0015  | 0.003       | 0.609                | 0.58                               | 0.58                                  |
| Q                                   | 0.0001   | 0.0001      | 0.189                |                                    |                                       |
| Forest                              | 0.0099   | 0.0096      | 0.326                |                                    |                                       |
| Water                               | 0.0068   | 0.0058      | 0.268                |                                    |                                       |
| T <sub>pref</sub> ~ W               | 0.0561   | 0.195       | 0.773                |                                    |                                       |
| Taverage                            | -0.0652  | 0.107       | 0.555                | 0.04                               | 0.04                                  |
| Q                                   | -0.0015  | 0.003       | 0.624                |                                    |                                       |
| Forest                              | -0.499   | 0.3545      | 0.1896               |                                    |                                       |
| Water                               | 0.107    | 0.2153      | 0.6301               |                                    |                                       |
| Taverage ~ Q                        | -0.8626  |             | <b>&lt;0.0001***</b> |                                    |                                       |
| TWL ~ CWL                           | 0.0218   |             | 0.404                |                                    |                                       |
| TWL ~ VO <sub>2</sub>               | 0.1201   |             | 0.088                |                                    |                                       |
| TWL ~ T <sub>pref</sub>             | 0.123    |             | 0.083                |                                    |                                       |
| VO <sub>2</sub> ~ CWL               | 0.256    |             | <b>0.0018**</b>      |                                    |                                       |
| VO <sub>2</sub> ~ T <sub>pref</sub> | 0.0588   |             | 0.255                |                                    |                                       |
| CWL ~ T <sub>pref</sub>             | -0.061   |             | 0.247                |                                    |                                       |
| Osmo ~ VO <sub>2</sub>              | -0.319   |             | <b>0.0001***</b>     |                                    |                                       |

Final path diagram with only significant paths showed:



| Population_name | Type                  | Altitude | Habitat_water | Habitat_forest | TPmean | Tpmin | Tpmax | Taverage | P      | Pcumulative | Pdry  | Pwet  | Q      |
|-----------------|-----------------------|----------|---------------|----------------|--------|-------|-------|----------|--------|-------------|-------|-------|--------|
| BETH            | Oviparous_PhyloFrance | 1054     | Permanent     | Shade          | 8.8    | -1.9  | 21.4  | 17.33    | 1389.5 | 682.8       | 84.6  | 140.9 | 305.82 |
| CORE            | Oviparous_PhyloFrance | 1405     | Periodic      | Open           | 8.75   | -1.9  | 21.3  | 17.33    | 1427.8 | 691.2       | 84.6  | 142.4 | 317.23 |
| CUG             | Oviparous_PhyloFrance | 488      | Permanent     | Shade          | 11.35  | 0     | 24.5  | 20.76    | 1045.2 | 517.1       | 70.6  | 107.1 | 174.13 |
| LERS            | Oviparous_PhyloFrance | 1246     | Permanent     | Open           | 8.15   | -3.8  | 22.4  | 18.16    | 1686.5 | 797.1       | 93.6  | 174.0 | 346.08 |
| PIAN            | Oviparous_PhyloFrance | 40       | Permanent     | Shade          | 13.3   | 2.9   | 26.4  | 22.70    | 933.2  | 401.5       | 53.8  | 106.6 | 135.53 |
| PED             | Oviparous_PhyloFrance | 370      | Permanent     | Open           | 11.35  | 0     | 24.2  | 20.85    | 1523.6 | 696.7       | 94.7  | 152.4 | 260.16 |
| BUZ             | Oviparous_PhyloFrance | 467      | Permanent     | Shade          | 12.25  | 0.8   | 24.9  | 21.70    | 1503.5 | 709.2       | 95.7  | 149.6 | 242.75 |
| GOUR            | Oviparous_PhyloFrance | 1224     | Periodic      | Open           | 7.85   | -1.9  | 20.4  | 16.56    | 1755.6 | 733.2       | 98.0  | 194.2 | 425.55 |
| MAR             | Oviparous_PhyloFrance | 990      | Periodic      | Shade          | 9.5    | -2.9  | 23.9  | 19.72    | 1199.3 | 569.7       | 67.8  | 128.2 | 213.09 |
| TUCO            | Oviparous_PhyloFrance | 76       | Periodic      | Shade          | 12.0   | 1.2   | 26.1  | 22.46    | 893.1  | 403.4       | 56.6  | 92.2  | 131.38 |
| BEN1            | Oviparous_PhyloFrance | 900      | Permanent     | Open           | 10.7   | -0.4  | 23.0  | 19.72    | 1774.4 | 782.3       | 104.3 | 178.3 | 335.53 |
| BEN2            | Oviparous_PhyloFrance | 900      | Periodic      | Open           | 9.5    | -1.3  | 21.6  | 18.12    | 1949.0 | 843.8       | 110.6 | 199.9 | 419.26 |
| ARB             | Oviparous_PhyloFrance | 465      | Periodic      | Shade          | 10.9   | -1.1  | 24.6  | 20.76    | 1122.8 | 562.3       | 71.0  | 118.7 | 185.91 |
| GOLF            | Oviparous_PhyloFrance | 40       | Permanent     | Shade          | 13.35  | 3.0   | 26.4  | 22.68    | 922.9  | 399.2       | 53.7  | 104.9 | 134.15 |
| MANI            | Oviparous_PhyloFrance | 66       | Periodic      | Shade          | 12.15  | 1.4   | 26.0  | 22.32    | 899.8  | 400.0       | 55.6  | 94.0  | 133.49 |
| CHIZE           | Muralis               | 64       | Periodic      | Open           | 12.25  | 2.3   | 26.0  | 22.13    | 868.0  | 367.8       | 49.0  | 98.6  | 129.41 |
| LEZ             | Viviparous            | 808      | Permanent     | Open           | 8.15   | -2.1  | 21.7  | 17.73    | 1445.1 | 634.8       | 82.2  | 154.3 | 309.79 |
| RODI            | Viviparous            | 808      | Permanent     | Open           | 8.3    | -2    | 21.9  | 17.88    | 1455.6 | 637.6       | 82.7  | 155.6 | 306.05 |

**Table S1:** Environmental data for sampled populations

**Table S2** : Mean morphological and physiological data by population.

| Type       | Population   | Population_N | SVL   | SD_SVL | BodyMass | SD_W | Surface | SD_surface | VO <sub>2</sub> | SD_VO <sub>2</sub> | T <sub>irref</sub> | SD_T <sub>irref</sub> | TEWL  | N_no_feces | SD_CEWL | SD_CEWL |      |
|------------|--|--------------|-------|--------|----------|------|---------|------------|-----------------|--------------------|--------------------|-----------------------|-------|------------|---------|---------|------|
| Oviparous  | Pian Médoc, Nouvelle-Aquitaine, France               | 10           | 52,00 | 3,86   | 2,50     | 0,54 | 19,11   | 3,03       | 0,171           | 0,027              | 33,88              | 2,45                  | 3,797 | 9          | 0,82    | 175,7   | 40,6 |
| Oviparous  | Landiras-Tuco-Nègues, Nouvelle-Aquitaine, France     | 12           | 49,17 | 4,24   | 2,05     | 0,47 | 16,97   | 2,79       | 0,141           | 0,027              | 33,87              | 2,57                  | 3,016 | 12         | 0,67    | 163,2   | 25,2 |
| Oviparous  | Tourbière de Pédestarrès, Nouvelle-Aquitaine, France | 10           | 51,70 | 1,49   | 2,42     | 0,25 | 17,69   | 2,03       | 0,17            | 0,02               | 34,98              | 2,07                  | 3,973 | 8          | 1,11    | 167,2   | 57,4 |
| Oviparous  | Buzy sud, Nouvelle-Aquitaine, France                 | 12           | 52,25 | 1,60   | 2,84     | 0,14 | 19,33   | 1,18       | 0,204           | 0,04               | 35,03              | 2,4                   | 4,200 | 11         | 0,75    | 189,4   | 34,0 |
| Oviparous  | Plateau du Bénou est, Nouvelle-Aquitaine, France     | 2            | 48,00 | 1,41   | 2,08     | 0,33 | 17,09   | 1,97       | 0,132           | 0,014              | 35,12              | 2,4                   | 3,190 | 2          | 0,48    | 159,9   | 41,1 |
| Oviparous  | Plateau du Bénou ouest, Nouvelle-Aquitaine, France   | 10           | 54,90 | 3,93   | 3,48     | 0,67 | 22,58   | 2,77       | 0,243           | 0,039              | 35,22              | 2,4                   | 3,735 | 10         | 0,41    | 181,9   | 25,7 |
| Oviparous  | Gourette, Nouvelle-Aquitaine, France                 | 8            | 55,88 | 1,46   | 3,42     | 0,35 | 21,81   | 2,26       | 0,251           | 0,038              | 34,93              | 2,33                  | 4,236 | 6          | 0,37    | 214,7   | 33,2 |
| Oviparous  | Col de Marrous, Occitanie, France                    | 12           | 50,00 | 3,54   | 2,48     | 0,52 | 19,27   | 2,96       | 0,175           | 0,037              | 35,09              | 1,96                  | 3,283 | 9          | 0,83    | 132,5   | 23,2 |
| Oviparous  | Etang de Lers, Occitanie, France                     | 10           | 52,80 | 2,25   | 2,67     | 0,24 | 20,34   | 1,28       | 0,207           | 0,026              | 35,41              | 2,71                  | 3,717 | 9          | 0,49    | 149,8   | 27,6 |
| Oviparous  | Cuguron, Occitanie, France                           | 12           | 51,75 | 2,86   | 2,69     | 0,36 | 19,54   | 1,69       | 0,21            | 0,047              | 34,82              | 2,44                  | 4,464 | 5          | 1,13    | 141,3   | 24,8 |
| Oviparous  | Col de la Core, Occitanie, France                    | 14           | 53,57 | 3,23   | 2,74     | 0,51 | 21,21   | 3,17       | 0,201           | 0,05               | 35,43              | 2,46                  | 3,111 | 12         | 0,52    | 173,5   | 21,7 |
| Oviparous  | Lac de Bethmale, Occitanie, France                   | 12           | 52,00 | 2,98   | 2,44     | 0,47 | 19,15   | 2,16       | 0,203           | 0,05               | 34,73              | 2,64                  | 3,738 | 4          | 0,63    | 173,5   | 26,5 |
| Oviparous  | Arbas, Occitanie, France                             | 9            | 48,78 | 3,70   | 2,15     | 0,43 | 18,32   | 1,92       | 0,161           | 0,033              | 34,72              | 2,19                  | 3,716 | 6          | 0,39    | 181,3   | 27,7 |
| Oviparous  | Landiras Manine, Nouvelle-Aquitaine, France          | 4            | 51,75 | 1,26   | 2,15     | 0,25 | 18,05   | 0,76       | 0,16            | 0,024              | 35,86              | 2,31                  | 3,179 | 2          | 0,12    | 164,6   | 11,1 |
| Oviparous  | Pian Médoc Golf, Nouvelle-Aquitaine, France          | 5            | 47,40 | 2,70   | 2,08     | 0,38 | 18,10   | 2,33       | 0,161           | 0,015              | 36,04              | 2,37                  | 3,072 | 5          | 0,31    | 206,3   | 40,5 |
| Viviparous | La Lézioux, Nouvelle-Aquitaine, France               | 11           | 53,73 | 4,05   | 2,99     | 0,65 | 21,64   | 3,15       | 0,23            | 0,057              | 35,22              | 2,24                  | 4,251 | 10         | 0,65    | 175,3   | 25,6 |
| Viviparous | La Lézioux Rodi, Nouvelle-Aquitaine, France          | 10           | 51,80 | 2,66   | 2,29     | 0,46 | 18,56   | 2,52       | 0,177           | 0,037              | 34,05              | 3,13                  | 3,615 | 8          | 0,57    | 180,3   | 20,3 |
| Muralis    | Chizé, Nouvelle-Aquitaine, France                    | 17           | 64,12 | 6,26   | 4,82     | 1,48 | NA      | NA         | 0,254           | 0,09               | 32,08              | 3,51                  | 4,083 | 16         | 1,70    | 106,0   | 20,1 |

**Table S3** : Snout-vent length model procedure and results

| Response              | Model                    | DF               | Log-likelihood     | AICc           | ΔAICc              | Weight |
|-----------------------|--------------------------|------------------|--------------------|----------------|--------------------|--------|
| Snout-vent length     | Age+temperature          | 5                | -310.12            | 630.67         | 0.00               | 0.26   |
|                       | Age+temperature+Forest   | 6                | -309.60            | 631.82         | 1.14               | 0.15   |
|                       | Age+Q+Forest             | 6                | -309.66            | 631.95         | 1.28               | 0.14   |
|                       | Age+temperature+Water    | 6                | -309.75            | 632.12         | 1.45               | 0.12   |
|                       | Age+Q                    | 5                | -311.10            | 632.63         | 1.96               | 0.10   |
|                       | Age+Q+Water              | 6                | -310.42            | 633.46         | 2.78               | 0.06   |
|                       | Age+Altitude             | 5                | -311.76            | 633.95         | 3.28               | 0.05   |
|                       | Age                      | 4                | -313.46            | 635.21         | 4.54               | 0.03   |
|                       | Age+Water                | 5                | -312.63            | 635.70         | 5.03               | 0.02   |
|                       | Age+Altitude+Forest      | 6                | -311.67            | 635.95         | 5.28               | 0.02   |
|                       | Age+precipitation        | 5                | -312.82            | 636.08         | 5.41               | 0.02   |
|                       | Age+precipitation+Water  | 6                | -311.81            | 636.24         | 5.57               | 0.02   |
|                       | Age+Forest               | 5                | -313.23            | 636.90         | 6.23               | 0.01   |
|                       | Age+Forest+Water         | 6                | -312.45            | 637.53         | 6.86               | 0.01   |
|                       | Age+Precipitation+Forest | 6                | -312.80            | 638.22         | 7.55               | 0.01   |
|                       | Q                        | 4                | -365.32            | 738.93         | 108.26             | 0.00   |
|                       | Q+Forest                 | 5                | -365.30            | 741.05         | 110.38             | 0.00   |
|                       | Q+Water                  | 5                | -365.31            | 741.07         | 110.40             | 0.00   |
|                       | Temperature              | 4                | -367.55            | 743.39         | 112.72             | 0.00   |
|                       | Temperature+Forest       | 5                | -366.71            | 743.87         | 113.19             | 0.00   |
|                       | Precipitation            | 4                | -368.35            | 744.99         | 114.32             | 0.00   |
|                       | Forest                   | 4                | -368.59            | 745.47         | 114.80             | 0.00   |
|                       | Temperature+Water        | 5                | -367.54            | 745.53         | 114.85             | 0.00   |
|                       | Precipitation+Forest     | 5                | -367.68            | 745.81         | 115.14             | 0.00   |
|                       | Altitude+Forest          | 5                | -367.71            | 745.86         | 115.19             | 0.00   |
|                       | Altitude                 | 4                | -369.01            | 746.32         | 115.65             | 0.00   |
|                       | Precipitation+Water      | 5                | -368.04            | 746.53         | 115.86             | 0.00   |
|                       | Forest+Water             | 5                | -368.54            | 747.52         | 116.85             | 0.00   |
| Altitude+Water        | 5                        | -369.01          | 748.45             | 117.78         | 0.00               |        |
| Water                 | 4                        | -371.63          | 751.55             | 120.88         | 0.00               |        |
| (conditional average) |                          |                  |                    |                |                    |        |
|                       | <b>Estimate</b>          | <b>Std.Error</b> | <b>Adjusted SE</b> | <b>z value</b> | <b>Pr(&gt; z )</b> |        |
| <i>(Intercept)</i>    | 49.7212756               | 0.3523980        | 0.3556643          | 139.798        | <2e-16             | ***    |
| <i>Ageclass</i>       | -3.2475412               | 0.2511359        | 0.2535277          | 12.809         | <2e-16             | ***    |
| <i>Temperature</i>    | -0.4555165               | 0.1640281        | 0.1796927          | 2.535          | 0.0112             | *      |
| <i>Forest</i>         | -0.5166520               | 0.5321827        | 0.5732784          | 0.901          | 0.3675             |        |
| <i>Q</i>              | 0.0110638                | 0.0051731        | 0.0055082          | 2.009          | 0.0446             | *      |
| <i>Water</i>          | 0.3225500                | 0.3254951        | 0.3587856          | 0.899          | 0.3686             |        |
| <i>Altitude</i>       | 0.0015327                | 0.0007865        | 0.0008684          | 1.765          | 0.0776             | .      |
| <i>Precipitation</i>  | 0.0031513                | 0.0026753        | 0.0029630          | 1.064          | 0.2875             |        |

**Table S4** : Body mass model procedure and results

| Response              | Model                    | DF               | Log-likelihood     | AICc           | ΔAICc              | Weight |
|-----------------------|--------------------------|------------------|--------------------|----------------|--------------------|--------|
| Body mass W           | SVL+Q+Forest             | 6                | -29.25             | 71.12          | 0.00               | 0.29   |
|                       | SVL+Q                    | 5                | -31.10             | 72.65          | 1.53               | 0.13   |
|                       | SVL+Altitude             | 5                | -31.28             | 73.01          | 1.89               | 0.11   |
|                       | SVL+Temperature          | 5                | -31.46             | 73.37          | 2.25               | 0.09   |
|                       | SVL+Precipitation        | 5                | -31.83             | 74.09          | 2.97               | 0.06   |
|                       | SVL+Altitude+Forest      | 6                | -31.05             | 74.73          | 3.61               | 0.05   |
|                       | SVL+Q+Water              | 6                | -31.09             | 74.81          | 3.70               | 0.05   |
|                       | SVL+Temperature+Forest   | 6                | -31.22             | 75.07          | 3.95               | 0.04   |
|                       | SVL+Precipitation+Forest | 6                | -31.27             | 75.16          | 4.04               | 0.04   |
|                       | SVL+Altitude+Water       | 6                | -31.28             | 75.19          | 4.07               | 0.04   |
|                       | SVL+Temperature+Water    | 6                | -31.46             | 75.54          | 4.43               | 0.03   |
|                       | SVL+Precipitation+Water  | 6                | -31.68             | 75.98          | 4.87               | 0.03   |
|                       | SVL                      | 4                | -33.85             | 76.00          | 4.88               | 0.02   |
|                       | SVL+Forest               | 5                | -33.50             | 77.44          | 6.33               | 0.01   |
|                       | SVL+Water                | 5                | -33.79             | 78.02          | 6.90               | 0.01   |
|                       | SVL+Forest+Water         | 6                | -33.46             | 79.54          | 8.42               | 0.00   |
|                       | Q                        | 4                | -108.01            | 224.32         | 153.20             | 0.00   |
|                       | Temperature              | 4                | -109.51            | 227.31         | 156.19             | 0.00   |
|                       | Altitude                 | 4                | -110.50            | 229.30         | 158.18             | 0.00   |
|                       | Precipitation            | 4                | -110.65            | 229.59         | 158.47             | 0.00   |
| Forest                | 4                        | -112.60          | 233.48             | 162.37         | 0.00               |        |
| Water                 | 4                        | -114.23          | 236.75             | 165.63         | 0.00               |        |
| (conditional average) |                          |                  |                    |                |                    |        |
|                       | <b>Estimate</b>          | <b>Std.Error</b> | <b>Adjusted SE</b> | <b>z value</b> | <b>Pr(&gt; z )</b> |        |
| <i>(Intercept)</i>    | -3.9355491               | 0.4120208        | 0.4159879          | 9.461          | <2e-16             | ***    |
| <i>SVL</i>            | 0.1316938                | 0.0079106        | 0.0079866          | 16.489         | <2e-16             | ***    |
| <i>Forest</i>         | -0.1287266               | 0.1015268        | 0.1088650          | 1.182          | 0.2370             |        |
| <i>Q</i>              | 0.0022352                | 0.0009875        | 0.0010504          | 2.128          | 0.0333             | *      |
| <i>Altitude</i>       | 0.0003030                | 0.0001299        | 0.0001432          | 2.115          | 0.0344             | *      |
| <i>Temperature</i>    | -0.0668759               | 0.0301300        | 0.0332202          | 2.013          | 0.0441             | *      |
| <i>Precipitation</i>  | 0.0009432                | 0.0004849        | 0.0005287          | 1.784          | 0.0744             | .      |
| <i>Water</i>          | 0.0075074                | 0.0582110        | 0.0643811          | 0.117          | 0.9072             |        |

**Table S5** : Body surface model procedure and results

| Response              | Model                  | DF               | Log-likelihood     | AICc           | ΔAICc              | Weight |     |
|-----------------------|------------------------|------------------|--------------------|----------------|--------------------|--------|-----|
| Body surface          | W+Forest+Q             | 6                | -875.78            | 1764.18        | 0.00               | 0.11   |     |
|                       | W                      | 4                | -877.95            | 1764.20        | 0.01               | 0.11   |     |
|                       | W+Q                    | 5                | -876.89            | 1764.22        | 0.04               | 0.11   |     |
|                       | W+Precipitation        | 5                | -876.91            | 1764.27        | 0.08               | 0.11   |     |
|                       | W+Q+Water              | 6                | -876.08            | 1764.79        | 0.60               | 0.08   |     |
|                       | W+Precipitation+Forest | 6                | -876.21            | 1765.04        | 0.86               | 0.07   |     |
|                       | W+Water                | 5                | -877.38            | 1765.19        | 1.01               | 0.07   |     |
|                       | W+Precipitation+Water  | 6                | -876.41            | 1765.44        | 1.26               | 0.06   |     |
|                       | W+Temperature          | 5                | -877.62            | 1765.68        | 1.50               | 0.05   |     |
|                       | W+Altitude             | 5                | -877.79            | 1766.01        | 1.83               | 0.05   |     |
|                       | W+Forest               | 5                | -877.91            | 1766.27        | 2.09               | 0.04   |     |
|                       | W+Temperature+Water    | 6                | -876.85            | 1766.32        | 2.13               | 0.04   |     |
|                       | W+Altitude+Water       | 6                | -877.10            | 1766.82        | 2.64               | 0.03   |     |
|                       | W+Water+Forest         | 6                | -877.32            | 1767.26        | 3.08               | 0.02   |     |
|                       | W+Temperature+Forest   | 6                | -877.59            | 1767.80        | 3.62               | 0.02   |     |
|                       | W+Altitude+Forest      | 6                | -877.79            | 1768.19        | 4.01               | 0.02   |     |
|                       | Q                      | 4                | -980.91            | 1970.12        | 205.93             | 0.00   |     |
|                       | Temperature            | 4                | -981.93            | 1972.14        | 207.96             | 0.00   |     |
|                       | Altitude               | 4                | -982.65            | 1973.59        | 209.41             | 0.00   |     |
|                       | Precipitation          | 4                | -983.95            | 1976.18        | 212.00             | 0.00   |     |
| Forest                | 4                      | -985.05          | 1978.40            | 214.22         | 0.00               |        |     |
| Water                 | 4                      | -987.03          | 1982.34            | 218.16         | 0.00               |        |     |
| (conditional average) |                        |                  |                    |                |                    |        |     |
|                       | <b>Estimate</b>        | <b>Std.Error</b> | <b>Adjusted SE</b> | <b>z value</b> | <b>Pr(&gt; z )</b> |        |     |
|                       | <i>(Intercept)</i>     | 766.23491        | 57.04815           | 57.57351       | 13.309             | <2e-16 | *** |
|                       | <i>Masse-W</i>         | 407.05079        | 19.12940           | 19.30324       | 21.087             | <2e-16 | *** |
|                       | <i>Forest</i>          | 21.22972         | 28.03036           | 29.96610       | 0.708              | 0.479  |     |
|                       | <i>Q</i>               | -0.36000         | 0.25073            | 0.26966        | 1.335              | 0.182  |     |
|                       | <i>Precipitation</i>   | -0.19135         | 0.13579            | 0.14776        | 1.295              | 0.195  |     |
|                       | <i>Water</i>           | 17.49224         | 14.71063           | 16.30333       | 1.073              | 0.283  |     |
|                       | <i>Temperature</i>     | 7.36643          | 8.44924            | 9.35061        | 0.788              | 0.431  |     |
|                       | <i>Altitude</i>        | -0.02266         | 0.03702            | 0.04098        | 0.553              | 0.580  |     |



**Tables S6 : Tail width model procedures and results**

| Response           | Model                        | DF      | Log-likelihood | AICc  | ΔAICc | Weight |
|--------------------|------------------------------|---------|----------------|-------|-------|--------|
| Maximum Tail width | SVL+Age+Forest+Altitude      | 7       | -39.932        | 95.2  | 0.00  | 0.624  |
|                    | SVL+Age+Altitude             | 6       | -42.312        | 97.6  | 2.42  | 0.186  |
|                    | SVL+Age+Altitude+Water       | 7       | -41.980        | 99.3  | 4.10  | 0.080  |
|                    | SVL+Age+Temperature+Forest   | 7       | -42.914        | 101.1 | 5.96  | 0.032  |
|                    | SVL+Age+Temperature          | 6       | -44.252        | 101.5 | 6.30  | 0.027  |
|                    | SVL+Age+Precipitation+Water  | 7       | -44.077        | 103.5 | 8.29  | 0.010  |
|                    | SVL+Age+Precipitation        | 6       | -45.270        | 103.5 | 8.34  | 0.010  |
|                    | SVL+Age+Precipitation+Forest | 7       | -44.114        | 103.5 | 8.37  | 0.010  |
|                    | SVL+Age+Q+Forest             | 7       | -44.504        | 104.3 | 9.14  | 0.006  |
|                    | SVL+Age+Q                    | 6       | -45.864        | 104.7 | 9.53  | 0.005  |
|                    | SVL+Age                      | 5       | -47.433        | 105.5 | 10.38 | 0.003  |
|                    | SVL+Age+Precipitation+Water  | 7       | -45.268        | 105.8 | 10.67 | 0.003  |
|                    | SVL+Age+Q+Water              | 7       | -45.834        | 107.0 | 11.81 | 0.002  |
|                    | SVL+Age+Forest               | 6       | -47.337        | 107.6 | 12.47 | 0.001  |
|                    | SVL+Age+Water                | 6       | -47.433        | 107.8 | 12.67 | 0.001  |
|                    | SVL+Age+Water+Forest         | 7       | -47.335        | 110.0 | 14.81 | 0.000  |
|                    | Altitude                     | 4       | -69.348        | 147.1 | 51.98 | 0.000  |
|                    | Temperature                  | 4       | -71.469        | 151.4 | 56.22 | 0.000  |
|                    | Precipitation                | 4       | -72.085        | 152.6 | 57.45 | 0.000  |
|                    | Q                            | 4       | -72.639        | 153.7 | 58.56 | 0.000  |
| Forest             | 4                            | -75.212 | 158.9          | 63.71 | 0.000 |        |
| Water              | 4                            | -76.297 | 161.0          | 65.88 | 0.000 |        |

(conditional average)

|               | Estimate   | Std.Error | Adjusted SE | z value | Pr(> z ) |     |
|---------------|------------|-----------|-------------|---------|----------|-----|
| (Intercept)   | 4.5211656  | 0.9849442 | 1.0003700   | 4.519   | 6.2e-06  | *** |
| SVL           | 0.0455255  | 0.0208723 | 0.0211989   | 2.148   | 0.031750 | *   |
| Age           | 0.5563833  | 0.1613219 | 0.1638186   | 3.396   | 0.000683 | *** |
| Forest        | -0.1693022 | 0.0817294 | 0.0915749   | 1.849   | 0.064489 | .   |
| Altitude      | 0.0006271  | 0.0001739 | 0.0001893   | 3.313   | 0.000923 | *** |
| Water         | -0.0435430 | 0.0626044 | 0.0700558   | 0.622   | 0.534239 |     |
| Temperature   | -0.1151752 | 0.0446805 | 0.0487634   | 2.362   | 0.018181 | *   |
| Precipitation | 0.0014785  | 0.0006805 | 0.0007409   | 1.996   | 0.045979 | *   |
| Q             | 0.0023807  | 0.0013984 | 0.0015058   | 1.581   | 0.113858 |     |

| Response                                     | Model                        | DF      | Log-likelihood | AICc  | ΔAICc | Weight |
|--|------------------------------|---------|----------------|-------|-------|--------|
| Tail width at<br>10th<br>subcaudal<br>scales | SVL+Age+Forest+Altitude      | 7       | -42.890        | 100.7 | 0.00  | 0.408  |
|  | SVL+Age+Altitude             | 6       | -44.168        | 101.1 | 0.31  | 0.350  |
|  | SVL+Age+Altitude+Water       | 7       | -44.056        | 103.1 | 2.33  | 0.127  |
|  | SVL+Age+temperature          | 6       | -46.353        | 105.4 | 4.68  | 0.039  |
|  | SVL+Age+temperature+Forest   | 7       | -45.671        | 106.3 | 5.56  | 0.025  |
|  | SVL+Age+Temperature+Water    | 7       | -46.311        | 107.6 | 6.84  | 0.013  |
|  | SVL+Age+Precipitation        | 6       | -47.886        | 108.5 | 7.75  | 0.008  |
|  | SVL+Age+Q                    | 6       | -48.023        | 108.8 | 8.02  | 0.007  |
|  | SVL+Age+Q+Forest             | 7       | -46.922        | 108.8 | 8.06  | 0.007  |
|  | SVL+Age+Precipitation+Forest | 7       | -47.265        | 109.5 | 8.75  | 0.005  |
|  | SVL+Age+Precipitation+Water  | 7       | -47.755        | 110.5 | 9.73  | 0.003  |
|  | SVL+Age+Q+Water              | 7       | -48.019        | 111.0 | 10.26 | 0.002  |
|  | SVL+Age                      | 5       | -50.647        | 111.8 | 11.06 | 0.002  |
|  | SVL+Age+Forest               | 6       | -50.199        | 113.1 | 12.37 | 0.001  |
|  | SVL+Age+Water                | 6       | -50.573        | 113.9 | 13.12 | 0.001  |
|  | SVL+Age+Water+Forest         | 7       | -50.162        | 115.3 | 14.54 | 0.000  |
|  | Altitude                     | 4       | -71.076        | 150.5 | 49.74 | 0.000  |
|  | Temperature                  | 4       | -72.248        | 152.8 | 52.09 | 0.000  |
|  | Q                            | 4       | -73.052        | 154.4 | 53.69 | 0.000  |
|  | Precipitation                | 4       | -74.323        | 157.0 | 56.24 | 0.000  |
| Forest                                       | 4                            | -76.878 | 162.1          | 61.35 | 0.000 |        |
| Water  | 4                            | -78.438 | 165.2          | 64.47 | 0.000 |        |

(conditional average)

|                      | Estimate   | Std.Error | Adjusted SE | z value | Pr(> z ) |     |
|----------------------|------------|-----------|-------------|---------|----------|-----|
| <i>(Intercept)</i>   | 1.1234696  | 0.7641251 | 0.7728306   | 1.454   | 0.146027 |     |
| <i>SVL</i>           | 0.0740024  | 0.0152182 | 0.0153916   | 4.808   | 1.5e-06  | *** |
| <i>Age</i>           | -0.0684483 | 0.0631020 | 0.0638127   | 1.073   | 0.283431 |     |
| <i>Forest</i>        | -0.0916487 | 0.0604674 | 0.0670204   | 1.367   | 0.171477 |     |
| <i>Altitude</i>      | 0.0004803  | 0.0001208 | 0.0001314   | 3.656   | 0.000256 | *** |
| <i>Water</i>         | -0.0191226 | 0.0481963 | 0.0534091   | 0.358   | 0.720314 |     |
| <i>Temperature</i>   | -0.0921790 | 0.0305810 | 0.0334608   | 2.755   | 0.005872 | **  |
| <i>Precipitation</i> | 0.0011319  | 0.0004912 | 0.0005354   | 2.114   | 0.034515 | *   |
| <i>Q</i>             | 0.0019655  | 0.0009665 | 0.0010370   | 1.895   | 0.058037 | .   |

**Table S7 : Total Water Loss (TWL) model procedure and results**

| Response              | Model                  | DF               | Log-likelihood     | AICc           | ΔAICc              | Weight  |     |
|-----------------------|------------------------|------------------|--------------------|----------------|--------------------|---------|-----|
| Total Water Loss TWL  | W+Water+Forest         | 6                | -100.95            | 214.71         | 0.00               | 0.21    |     |
|                       | W+Altitude+Water       | 6                | -101.08            | 214.98         | 0.27               | 0.18    |     |
|                       | W+Water                | 5                | -102.21            | 214.99         | 0.28               | 0.18    |     |
|                       | W+Q+Water              | 6                | -101.30            | 215.42         | 0.71               | 0.15    |     |
|                       | W+Temperature+Water    | 6                | -101.54            | 215.89         | 1.18               | 0.12    |     |
|                       | W+Precipitation+Water  | 6                | -101.69            | 216.19         | 1.48               | 0.10    |     |
|                       | W+Altitude             | 5                | -105.04            | 220.66         | 5.95               | 0.01    |     |
|                       | W                      | 4                | -106.27            | 220.91         | 6.20               | 0.01    |     |
|                       | W+Temperature          | 5                | -105.28            | 221.13         | 6.42               | 0.01    |     |
|                       | W+Forest               | 5                | -105.39            | 221.36         | 6.66               | 0.01    |     |
|                       | W+Q                    | 5                | -105.49            | 221.55         | 6.84               | 0.01    |     |
|                       | W+Altitude+Forest      | 6                | -104.91            | 222.63         | 7.92               | 0.00    |     |
|                       | W+Precipitation        | 5                | -106.10            | 222.77         | 8.06               | 0.00    |     |
|                       | W+Temperature+Forest   | 6                | -105.12            | 223.05         | 8.34               | 0.00    |     |
|                       | W+Precipitation+Forest | 6                | -105.21            | 223.24         | 8.53               | 0.00    |     |
|                       | W+Q+Forest             | 6                | -105.34            | 223.50         | 8.79               | 0.00    |     |
|                       | Water                  | 4                | -121.18            | 250.73         | 36.02              | 0.00    |     |
|                       | Precipitation          | 4                | -121.57            | 251.51         | 36.80              | 0.00    |     |
|                       | Q                      | 4                | -121.93            | 252.23         | 37.52              | 0.00    |     |
|                       | Temperature            | 4                | -122.33            | 253.04         | 38.33              | 0.00    |     |
| Forest                | 4                      | -122.44          | 253.27             | 38.56          | 0.00               |         |     |
| Altitude              | 4                      | -122.45          | 253.28             | 38.57          | 0.00               |         |     |
| (conditional average) |                        |                  |                    |                |                    |         |     |
|                       | <b>Estimate</b>        | <b>Std.Error</b> | <b>Adjusted SE</b> | <b>z value</b> | <b>Pr(&gt; z )</b> |         |     |
|                       | <i>(Intercept)</i>     | 1.5861507        | 0.3209197          | 0.3249125      | 4.882              | 1.1e-06 | *** |
|                       | <i>Masse-W</i>         | 0.0007870        | 0.0001193          | 0.0001208      | 6.514              | <0.0001 | *** |
|                       | <i>Water</i>           | -0.2586069       | 0.0730132          | 0.0808788      | 3.197              | 0.00139 | **  |
|                       | <i>Forest</i>          | -0.1218857       | 0.0821922          | 0.0910608      | 1.339              | 0.18073 |     |
|                       | <i>Altitude</i>        | -0.0002560       | 0.0001655          | 0.0001836      | 1.394              | 0.16318 |     |
|                       | <i>Q</i>               | -0.0011482       | 0.0008745          | 0.0009707      | 1.183              | 0.23685 |     |
|                       | <i>Temperature</i>     | 0.0481145        | 0.0409312          | 0.0453551      | 1.061              | 0.28876 |     |
|                       | <i>Precipitation</i>   | -0.0005166       | 0.0005990          | 0.0006591      | 0.784              | 0.43317 |     |

**Table S8** : Cutaneous Water Loss (CEWL) model procedure and results

| Response              | Model                         | DF               | Log-likelihood     | AICc           | ΔAICc              | Weight |
|-----------------------|-------------------------------|------------------|--------------------|----------------|--------------------|--------|
| CEWL                  | Humidity                      | 5                | -1389.29           | 2788.69        | 0.00               | 0.22   |
|                       | Humidity+Q                    | 6                | -1389.07           | 2790.31        | 1.62               | 0.10   |
|                       | Humidity+Altitude             | 6                | -1389.18           | 2790.52        | 1.83               | 0.09   |
|                       | Humidity+Precipitation        | 6                | -1389.25           | 2790.67        | 1.98               | 0.08   |
|                       | Humidity+Forest               | 6                | -1389.28           | 2790.72        | 2.03               | 0.08   |
|                       | Humidity+Temperature          | 6                | -1389.28           | 2790.73        | 2.04               | 0.08   |
|                       | Humidity+Water                | 6                | -1389.29           | 2790.74        | 2.04               | 0.08   |
|                       | Humidity+Q+Forest             | 7                | -1388.84           | 2791.90        | 3.21               | 0.04   |
|                       | Humidity+Altitude+Forest      | 7                | -1389.04           | 2792.31        | 3.62               | 0.04   |
|                       | Humidity+Q+Water              | 7                | -1389.06           | 2792.35        | 3.66               | 0.03   |
|                       | Humidity+Altitude+Water       | 7                | -1389.18           | 2792.58        | 3.88               | 0.03   |
|                       | Humidity+Precipitation+Forest | 7                | -1389.25           | 2792.71        | 4.02               | 0.03   |
|                       | Humidity+Precipitation+Water  | 7                | -1389.25           | 2792.73        | 4.03               | 0.03   |
|                       | Humidity+Forest+Water         | 7                | -1389.28           | 2792.78        | 4.08               | 0.03   |
|                       | Humidity+Temperature+Forest   | 7                | -1389.28           | 2792.78        | 4.09               | 0.03   |
|                       | Humidity+Temperature+Water    | 7                | -1389.28           | 2792.78        | 4.09               | 0.03   |
|                       | Q                             | 5                | -1395.57           | 2801.26        | 12.57              | 0.00   |
|                       | Forest                        | 5                | -1395.89           | 2801.90        | 13.21              | 0.00   |
|                       | Temperature                   | 5                | -1396.01           | 2802.14        | 13.45              | 0.00   |
|                       | Precipitation                 | 5                | -1396.02           | 2802.15        | 13.46              | 0.00   |
| Water                 | 5                             | -1396.04         | 2802.19            | 13.50          | 0.00               |        |
| Altitude              | 5                             | -1396.04         | 2802.20            | 13.51          | 0.00               |        |
| (conditional average) |                               |                  |                    |                |                    |        |
|                       | <b>Estimate</b>               | <b>Std.Error</b> | <b>Adjusted SE</b> | <b>z value</b> | <b>Pr(&gt; z )</b> |        |
| <i>(Intercept)</i>    | 9.7237649                     | 1.9835767        | 1.9898381          | 4.887          | 1,00E-06           | ***    |
| <i>Humidity</i>       | 0.6998795                     | 0.1799873        | 0.1805695          | 3.876          | 0.000106           | ***    |
| <i>Q</i>              | 0.0041834                     | 0.0059314        | 0.0065127          | 0.642          | 0.520649           |        |
| <i>Altitude</i>       | -0.0005571                    | 0.0010761        | 0.0011886          | 0.469          | 0.639278           |        |
| <i>Precipitation</i>  | 0.0008976                     | 0.0035409        | 0.0039203          | 0.229          | 0.818892           |        |
| <i>Forest</i>         | -0.0291128                    | 0.6704440        | 0.7331314          | 0.040          | 0.968324           |        |
| <i>Temperature</i>    | -0.0156274                    | 0.2427493        | 0.2686752          | 0.058          | 0.953617           |        |
| <i>Water</i>          | -0.0285093                    | 0.4732719        | 0.5244952          | 0.054          | 0.956652           |        |

**Table S9 : Oxygen consumption (VO2) model procedure and results**

| Response              | Model                  | DF               | Log-likelihood     | AICc           | ΔAICc              | Weight |     |
|-----------------------|------------------------|------------------|--------------------|----------------|--------------------|--------|-----|
|                       | W+Q+Forest             | 6                | 293.26             | -573.87        | 0.00               | 0.11   |     |
| VO2                   | W+Temperature+Water    | 6                | 293.18             | -573.72        | 0.15               | 0.10   |     |
|                       | W                      | 4                | 290.99             | -573.68        | 0.19               | 0.10   |     |
|                       | W+Water                | 5                | 291.89             | -573.32        | 0.55               | 0.08   |     |
|                       | W+Temperature          | 5                | 291.75             | -573.04        | 0.83               | 0.07   |     |
|                       | W+Q+Water              | 6                | 292.79             | -572.92        | 0.95               | 0.07   |     |
|                       | W+Q                    | 5                | 291.66             | -572.86        | 1.00               | 0.07   |     |
|                       | W+Precipitation        | 5                | 291.62             | -572.78        | 1.08               | 0.06   |     |
|                       | W+Precipitation+Forest | 6                | 292.62             | -572.60        | 1.27               | 0.06   |     |
|                       | W+Altitude+Water       | 6                | 292.52             | -572.39        | 1.48               | 0.05   |     |
|                       | W+Temperature+Forest   | 6                | 292.43             | -572.22        | 1.65               | 0.05   |     |
|                       | W+Precipitation+Water  | 6                | 292.39             | -572.13        | 1.73               | 0.05   |     |
|                       | W+Altitude             | 5                | 291.29             | -572.11        | 1.76               | 0.05   |     |
|                       | W+Forest               | 5                | 291.04             | -571.62        | 2.25               | 0.04   |     |
|                       | W+Forest+Water         | 6                | 291.90             | -571.15        | 2.72               | 0.03   |     |
|                       | W+Altitude+Forest      | 6                | 291.62             | -570.58        | 3.29               | 0.02   |     |
|                       | Q                      | 4                | 246.98             | -485.66        | 88.21              | 0.00   |     |
|                       | Temperature            | 4                | 245.59             | -482.87        | 91.00              | 0.00   |     |
|                       | Precipitation          | 4                | 244.30             | -480.30        | 93.57              | 0.00   |     |
|                       | Altitude               | 4                | 243.93             | -479.55        | 94.32              | 0.00   |     |
|                       | Forest                 | 4                | 241.88             | -475.45        | 98.42              | 0.00   |     |
| Water                 | 4                      | 240.43           | -472.56            | 101.31         | 0.00               |        |     |
| (conditional average) |                        |                  |                    |                |                    |        |     |
|                       | <b>Estimate</b>        | <b>Std.Error</b> | <b>Adjusted SE</b> | <b>z value</b> | <b>Pr(&gt; z )</b> |        |     |
|                       | <i>(Intercept)</i>     | 3.131e-02        | 1.321e-02          | 1.333e-02      | 2.348              | 0.0189 | *   |
|                       | <i>Masse-W</i>         | 6.150e-05        | 5.018e-06          | 5.064e-06      | 12.144             | <2e-16 | *** |
|                       | <i>Forest</i>          | -4.413e-03       | 4.229e-03          | 4.551e-03      | 0.970              | 0.3323 |     |
|                       | <i>Q</i>               | 6.508e-05        | 4.817e-05          | 5.167e-05      | 1.259              | 0.2079 |     |
|                       | <i>Water</i>           | -3.599e-03       | 2.494e-03          | 2.761e-03      | 1.304              | 0.1923 |     |
|                       | <i>Temperature</i>     | -2.130e-03       | 1.468e-03          | 1.619e-03      | 1.316              | 0.1883 |     |
|                       | <i>Precipitation</i>   | 3.043e-05        | 2.538e-05          | 2.755e-05      | 1.104              | 0.2694 |     |
|                       | <i>Altitude</i>        | 6.095e-06        | 6.269e-06          | 6.927e-06      | 0.880              | 0.3789 |     |

**Table S10 : Osmolality model procedure and results**

| Response              | Model                  | DF               | Log-likelihood     | AICc           | ΔAICc              | Weight |
|-----------------------|------------------------|------------------|--------------------|----------------|--------------------|--------|
| Osmolality            | W                      | 4                | -571.026           | 1150.4         | 0.00               | 0.084  |
|                       | Altitude               | 4                | -571.097           | 1150.5         | 0.14               | 0.078  |
|                       | Temperature            | 4                | -571.117           | 1150.5         | 0.18               | 0.077  |
|                       | Water                  | 4                | -571.339           | 1151.0         | 0.63               | 0.061  |
|                       | Forest                 | 4                | -571.356           | 1151.0         | 0.66               | 0.060  |
|                       | Q                      | 4                | -571.358           | 1151.0         | 0.66               | 0.060  |
|                       | Precipitation          | 4                | -571.474           | 1151.3         | 0.90               | 0.054  |
|                       | Altitude+Water         | 5                | -570.789           | 1152.0         | 1.68               | 0.036  |
|                       | Temperature+water      | 5                | -570.797           | 1152.1         | 1.70               | 0.036  |
|                       | W+Water                | 5                | -570.822           | 1152.1         | 1.75               | 0.035  |
|                       | W+Altitude             | 5                | -570.829           | 1152.1         | 1.76               | 0.035  |
|                       | W+Temperature          | 5                | -570.856           | 1152.2         | 1.82               | 0.034  |
|                       | W+Forest               | 5                | -570.979           | 1152.4         | 2.06               | 0.030  |
|                       | W+Q                    | 5                | -571.010           | 1152.5         | 2.13               | 0.029  |
|                       | W+Precipitation        | 5                | -571.014           | 1152.5         | 2.13               | 0.029  |
|                       | Altitude+Forest        | 5                | -571.097           | 1152.7         | 2.30               | 0.027  |
|                       | Temperature+Forest     | 5                | -571.116           | 1152.7         | 2.34               | 0.026  |
|                       | Q+Water                | 5                | -571.163           | 1152.8         | 2.43               | 0.025  |
|                       | Water+Forest           | 5                | -571.170           | 1152.8         | 2.45               | 0.025  |
|                       | Precipitation+Forest   | 5                | -571.288           | 1153.0         | 2.68               | 0.022  |
|                       | Precipitation+Water    | 5                | -571.332           | 1153.1         | 2.77               | 0.021  |
|                       | Q+Forest               | 5                | -571.343           | 1153.2         | 2.79               | 0.021  |
|                       | W+Temperature+Water    | 6                | -570.527           | 1153.7         | 3.35               | 0.016  |
|                       | W+Water+Forest         | 6                | -570.748           | 1154.2         | 3.79               | 0.013  |
|                       | W+Q+Water              | 6                | -570.786           | 1154.2         | 3.87               | 0.012  |
|                       | W+Precipitation+Water  | 6                | -570.802           | 1154.3         | 3.90               | 0.012  |
|                       | W+Precipitation+Forest | 6                | -570.822           | 1154.3         | 3.94               | 0.012  |
|                       | W+Altitude+Forest      | 6                | -570.828           | 1154.3         | 3.95               | 0.012  |
| W+Temperature+Forest  | 6                      | -570.855         | 1154.4             | 4.01           | 0.011              |        |
| W+Q+Forest            | 6                      | -570.974         | 1154.6             | 4.25           | 0.010              |        |
| (conditional average) |                        |                  |                    |                |                    |        |
|                       | <b>Estimate</b>        | <b>Std.Error</b> | <b>Adjusted SE</b> | <b>z value</b> | <b>Pr(&gt; z )</b> |        |
| <i>(Intercept)</i>    | 309.378473             | 6.508595         | 6.557578           | 47.179         | <2e-16             | ***    |
| <i>Masse-W</i>        | -2.399728              | 2.769019         | 2.797445           | 0.858          | 0.391              |        |
| <i>Altitude</i>       | -0.004967              | 0.006052         | 0.006686           | 0.743          | 0.458              |        |
| <i>Temperature</i>    | 1.077801               | 1.374722         | 1.519391           | 0.709          | 0.478              |        |
| <i>Water</i>          | 1.733378               | 2.654308         | 2.939767           | 0.590          | 0.555              |        |
| <i>Forest</i>         | -0.992380              | 3.485592         | 3.846084           | 0.258          | 0.796              |        |
| <i>Q</i>              | -0.010045              | 0.032601         | 0.036017           | 0.279          | 0.780              |        |
| <i>Precipitation</i>  | 0.002367               | 0.021499         | 0.023658           | 0.100          | 0.920              |        |

**Table S11 : Thermal preference model procedure and results**

| Response    | Model                         | DF       | Log-likelihood | AICc    | ΔAICc | Weight |
|-------------|-------------------------------|----------|----------------|---------|-------|--------|
| Tpref       | Bac+Time+Forest               | 20       | -3252.98       | 6546.55 | 0.00  | 0.12   |
|             | Bac+Time                      | 19       | -3254.12       | 6546.78 | 0.23  | 0.11   |
|             | Bac+Time+Altitude             | 20       | -3253.23       | 6547.06 | 0.51  | 0.10   |
|             | Bac+Time+Precipitation        | 20       | -3253.30       | 6547.19 | 0.64  | 0.09   |
|             | Bac+Time+Q                    | 20       | -3253.44       | 6547.47 | 0.92  | 0.08   |
|             | Bac+Time+Temperature          | 20       | -3253.60       | 6547.78 | 1.23  | 0.07   |
|             | Bac+Time+Altitude+Forest      | 21       | -3252.77       | 6548.18 | 1.63  | 0.05   |
|             | Bac+Time+Precipitation+Forest | 21       | -3252.92       | 6548.50 | 1.95  | 0.05   |
|             | Bac+Time+Temperature+Forest   | 21       | -3252.96       | 6548.57 | 2.02  | 0.05   |
|             | Bac+Time+Forest+Water         | 21       | -3252.96       | 6548.57 | 2.02  | 0.05   |
|             | Bac+Time+Q+Forest             | 21       | -3252.98       | 6548.61 | 2.06  | 0.04   |
|             | Bac+Time+Forest               | 20       | -3254.12       | 6548.83 | 2.28  | 0.04   |
|             | Bac+Time+Altitude+Water       | 21       | -3253.18       | 6549.02 | 2.46  | 0.04   |
|             | Bac+Time+Precipitation+Water  | 21       | -3253.30       | 6549.25 | 2.69  | 0.03   |
|             | Bac+Time+Q+Water              | 21       | -3253.42       | 6549.49 | 2.94  | 0.03   |
|             | Bac+Time+Temperature+Water    | 21       | -3253.56       | 6549.77 | 3.21  | 0.02   |
|             | Altitude                      | 5        | -3270.88       | 6551.81 | 5.25  | 0.01   |
|             | Precipitation                 | 5        | -3271.01       | 6552.07 | 5.51  | 0.01   |
|             | Forest                        | 5        | -3271.01       | 6552.07 | 5.51  | 0.01   |
|             | Q                             | 5        | -3271.19       | 6552.43 | 5.88  | 0.01   |
| Temperature | 5                             | -3271.32 | 6552.68        | 6.13    | 0.01  |        |
| Water       | 5                             | -3272.10 | 6554.24        | 7.68    | 0.00  |        |

(conditional average)

|               | Estimate   | Std.Error | Adjusted SE | z value | Pr(> z ) |     |
|---------------|------------|-----------|-------------|---------|----------|-----|
| (Intercept)   | 34.2803458 | 0.4323040 | 0.4326677   | 79.230  | <0.0001  | *** |
| Bac10         | 0.8485382  | 0.4632778 | 0.4683304   | 1.812   | 0.070011 | .   |
| Bac11         | 1.1221163  | 0.4652734 | 0.4703473   | 2.386   | 0.017046 | *   |
| Bac12         | 1.2471343  | 0.4693188 | 0.4744375   | 2.629   | 0.008572 | **  |
| Bac13         | 0.2281367  | 0.4958455 | 0.5012498   | 0.455   | 0.649011 |     |
| Bac14         | 1.0831152  | 0.4915306 | 0.4968835   | 2.180   | 0.029271 | *   |
| Bac15         | 1.3346968  | 0.4889626 | 0.4942855   | 2.700   | 0.006929 | **  |
| Bac2          | 1.2393527  | 0.4682831 | 0.4733978   | 2.618   | 0.008845 | **  |
| Bac3          | 1.0456786  | 0.4709682 | 0.4761117   | 2.196   | 0.028071 | *   |
| Bac4          | 1.2337184  | 0.4693900 | 0.4745165   | 2.600   | 0.009324 | **  |
| Bac5          | 1.6075773  | 0.4805428 | 0.4857912   | 3.309   | 0.000936 | *** |
| Bac6          | 0.4031250  | 0.4747964 | 0.4799812   | 0.840   | 0.400978 |     |
| Bac7          | -0.0157553 | 0.4461975 | 0.4510618   | 0.035   | 0.972136 |     |
| Bac8          | 1.1169317  | 0.4544411 | 0.4593951   | 2.431   | 0.015044 | *   |
| Bac9          | 1.6338977  | 0.4611215 | 0.4661417   | 3.505   | 0.000456 | *** |
| Time          | -0.0518281 | 0.0561136 | 0.0561661   | 0.923   | 0.356130 |     |
| Forest        | 0.1988738  | 0.1632889 | 0.1807869   | 1.100   | 0.271312 |     |
| Altitude      | 0.0003645  | 0.0003041 | 0.0003342   | 1.091   | 0.275440 |     |
| Precipitation | 0.0010364  | 0.0010656 | 0.0011676   | 0.888   | 0.374724 |     |
| Q             | 0.0012692  | 0.0017988 | 0.0019609   | 0.647   | 0.517462 |     |
| Temperature   | -0.0554786 | 0.0746084 | 0.0816734   | 0.679   | 0.496965 |     |
| Water         | -0.0205471 | 0.1377719 | 0.1526137   | 0.135   | 0.892901 |     |

**Tables S12** : Morphology with extended data on oviparous males

| Response              | Model                    | DF               | Log-likelihood     | AICc           | ΔAICc              | Weight |
|-----------------------|--------------------------|------------------|--------------------|----------------|--------------------|--------|
| SVL                   | Age+Precipitation        | 5                | -1415.651          | 2841.4         | 0.00               | 0.297  |
|                       | Age+Altitude             | 5                | -1416.210          | 2842.5         | 1.12               | 0.170  |
|                       | Age+Precipitation+Forest | 6                | -1415.530          | 2843.2         | 1.81               | 0.120  |
|                       | Age+Precipitation+Water  | 6                | -1415.625          | 2843.4         | 2.00               | 0.109  |
|                       | Age+Altitude+Forest      | 6                | -1415.765          | 2843.7         | 2.28               | 0.095  |
|                       | Age+Q                    | 5                | -1417.203          | 2844.5         | 3.10               | 0.063  |
|                       | Age+Temperature          | 5                | -1417.354          | 2844.8         | 3.41               | 0.054  |
|                       | Age+Q+Water              | 6                | -1417.133          | 2846.4         | 5.01               | 0.024  |
|                       | Age+Q+Forest             | 6                | -1417.139          | 2846.5         | 5.03               | 0.024  |
|                       | Age+Temperature+Forest   | 6                | -1417.319          | 2846.8         | 5.39               | 0.020  |
|                       | Age+Temperature+Water    | 6                | -1417.337          | 2846.8         | 5.42               | 0.020  |
|                       | Age                      | 4                | -1421.639          | 2851.4         | 9.93               | 0.002  |
|                       | Age+Forest               | 5                | -1421.476          | 2853.1         | 11.65              | 0.001  |
|                       | Age+Water                | 5                | -1421.638          | 2853.4         | 11.97              | 0.001  |
|                       | Age+Water+Forest         | 6                | -1421.465          | 2855.1         | 13.68              | 0.000  |
|                       | Altitude+Forest          | 5                | -1679.355          | 3368.8         | 527.41             | 0.000  |
|                       | Precipitation+Forest     | 5                | -1679.974          | 3370.1         | 528.65             | 0.000  |
|                       | Precipitation            | 4                | -1681.138          | 3370.4         | 528.93             | 0.000  |
|                       | Altitude                 | 4                | -1681.483          | 3371.0         | 529.62             | 0.000  |
|                       | Precipitation+Water      | 5                | -1680.973          | 3372.1         | 530.64             | 0.000  |
|                       | Altitude+Water           | 5                | -1681.423          | 3373.0         | 531.54             | 0.000  |
|                       | Q                        | 4                | -1682.734          | 3373.6         | 532.12             | 0.000  |
|                       | Temperature              | 4                | -1682.744          | 3373.6         | 532.14             | 0.000  |
|                       | Q+Forest                 | 5                | -1681.815          | 3373.8         | 532.33             | 0.000  |
|                       | Temperature+Forest       | 5                | -1681.909          | 3373.9         | 532.52             | 0.000  |
|                       | Q+Water                  | 5                | -1682.490          | 3375.1         | 533.68             | 0.000  |
| Temperature+Water     | 5                        | -1682.596        | 3375.3             | 533.89         | 0.000              |        |
| Forest                | 4                        | -1685.793        | 3379.7             | 538.24         | 0.000              |        |
| Water                 | 4                        | -1685.809        | 3379.7             | 538.27         | 0.000              |        |
| Water+Forest          | 5                        | -1685.742        | 3381.6             | 540.18         | 0.000              |        |
| (conditional average) |                          |                  |                    |                |                    |        |
|                       | <b>Estimate</b>          | <b>Std.Error</b> | <b>Adjusted SE</b> | <b>z value</b> | <b>Pr(&gt; z )</b> |        |
| <i>(Intercept)</i>    | 48.9827392               | 0.3923401        | 0.3934680          | 124.490        | <2e-16             | ***    |
| <i>Age</i>            | 6.4359591                | 0.2056705        | 0.2062649          | 31.202         | <2e-16             | ***    |
| <i>Precipitation</i>  | -0.0132069               | 0.0036875        | 0.0037566          | 3.516          | 0.000439           | ***    |
| <i>Altitude</i>       | -0.0031770               | 0.0009474        | 0.0009501          | 3.344          | 0.000826           | ***    |
| <i>Forest</i>         | 0.2612238                | 0.4325255        | 0.4400898          | 0.594          | 0.552800           |        |
| <i>Water</i>          | -0.0970717               | 0.3987789        | 0.4063132          | 0.239          | 0.811177           |        |
| <i>Q</i>              | -0.0153039               | 0.0050396        | 0.0051342          | 2.981          | 0.002875           | **     |
| <i>Temperature</i>    | 0.6195826                | 0.2079668        | 0.2118733          | 2.924          | 0.003452           | **     |



| Response              | Model                    | DF       | Log-likelihood | AICc    | ΔAICc   | Weight |
|-----------------------|--------------------------|----------|----------------|---------|---------|--------|
| Body Mass (all males) | SVL+Altitude             | 5        | -147.902       | 305.9   | 0.00    | 0.414  |
|                       | SVL+Altitude+Water       | 6        | -146.972       | 306.1   | 0.19    | 0.376  |
|                       | SVL+Altitude+Forest      | 6        | -147.591       | 307.4   | 1.43    | 0.203  |
|                       | SVL+Temperature+Forest   | 6        | -151.860       | 315.9   | 9.97    | 0.003  |
|                       | SVL+Temperature          | 5        | -153.541       | 317.2   | 11.28   | 0.001  |
|                       | SVL+Temperature+water    | 6        | -153.023       | 318.2   | 12.29   | 0.001  |
|                       | SVL+Q+Forest             | 6        | -153.259       | 318.7   | 12.76   | 0.001  |
|                       | SVL+Forest               | 5        | -154.706       | 319.5   | 13.61   | 0.000  |
|                       | SVL+Precipitation+Forest | 6        | -154.073       | 320.3   | 14.39   | 0.000  |
|                       | SVL+Q                    | 5        | -155.142       | 320.4   | 14.48   | 0.000  |
|                       | SVL+Water+Forest         | 6        | -154.515       | 321.2   | 15.28   | 0.000  |
|                       | SVL+Q+Water              | 6        | -154.745       | 321.7   | 15.74   | 0.000  |
|                       | SVL+Precipitation        | 5        | -156.313       | 322.8   | 16.82   | 0.000  |
|                       | SVL+Precipitation+Water  | 6        | -155.768       | 323.7   | 17.78   | 0.000  |
|                       | SVL                      | 4        | -157.939       | 324.0   | 18.03   | 0.000  |
|                       | SVL+Water                | 5        | -157.296       | 324.7   | 18.79   | 0.000  |
|                       | Precipitation            | 4        | -702.783       | 1413.6  | 1107.72 | 0.000  |
|                       | Q                        | 4        | -704.193       | 1416.5  | 1110.54 | 0.000  |
|                       | Forest                   | 4        | -704.511       | 1417.1  | 1111.18 | 0.000  |
| Temperature           | 4                        | -704.521 | 1417.1         | 1111.20 | 0.000   |        |
| Altitude              | 4                        | -704.623 | 1417.3         | 1111.40 | 0.000   |        |
| Water                 | 4                        | -705.064 | 1418.2         | 1112.28 | 0.000   |        |

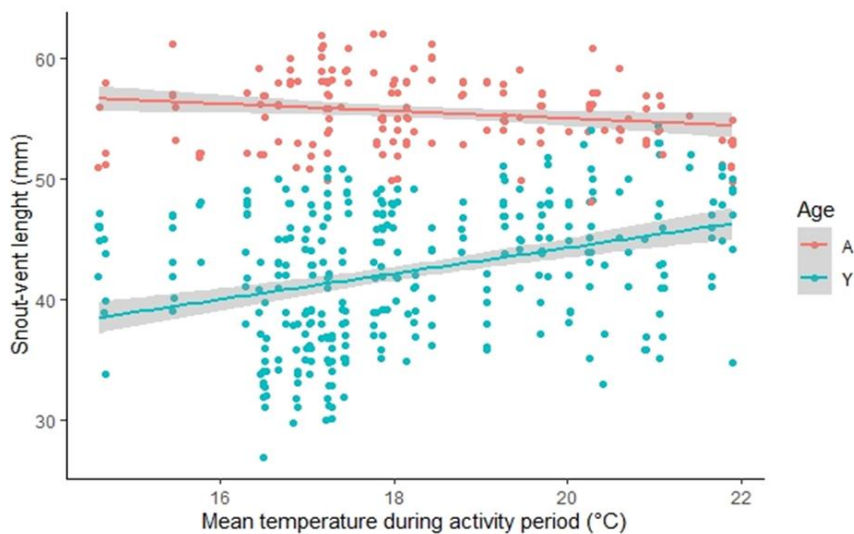
(conditional average)

|                      | Estimate   | Std.Error | Adjusted SE | z value | Pr(> z ) |     |
|----------------------|------------|-----------|-------------|---------|----------|-----|
| <i>(Intercept)</i>   | -3.877e+00 | 9.558e-02 | 9.586e-02   | 40.441  | <2e-16   | *** |
| <i>SVL</i>           | 1.288e-01  | 1.976e-03 | 1.982e-03   | 65.004  | <2e-16   | *** |
| <i>Altitude</i>      | 2.598e-04  | 5.702e-05 | 5.719e-05   | 4.543   | 5.5e-06  | *** |
| <i>Water</i>         | -3.273e-02 | 2.401e-02 | 2.446e-02   | 1.338   | 0.1809   |     |
| <i>Forest</i>        | 2.151e-02  | 2.692e-02 | 2.740e-02   | 0.785   | 0.4325   |     |
| <i>Temperature</i>   | -3.679e-02 | 1.422e-02 | 1.447e-02   | 2.542   | 0.0110   | *   |
| <i>Q</i>             | 6.630e-04  | 3.535e-04 | 3.597e-04   | 1.843   | 0.0653   | .   |
| <i>Precipitation</i> | 3.443e-04  | 2.690e-04 | 2.737e-04   | 1.258   | 0.2084   |     |

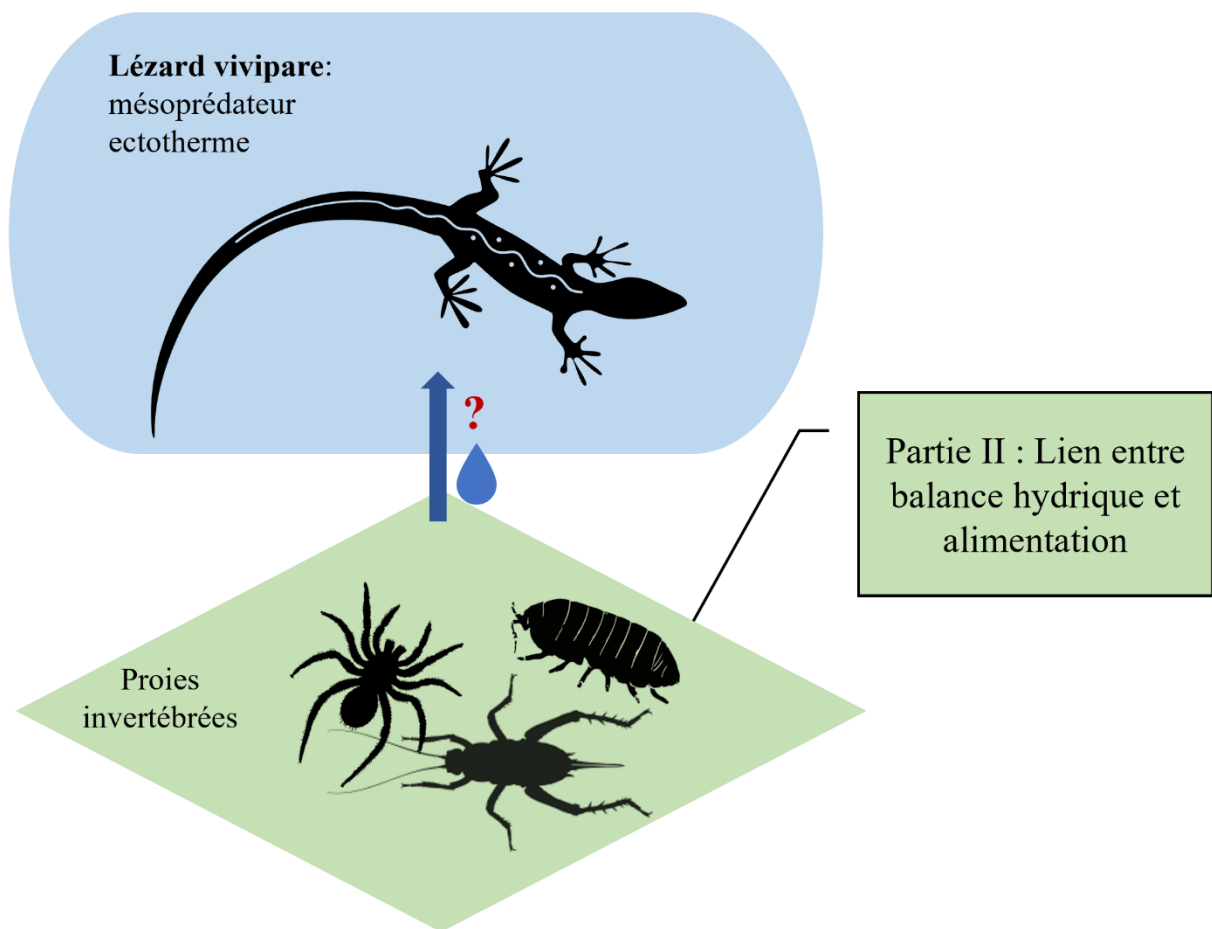
| Response           | Model                    | DF       | Log-likelihood | AICc   | ΔAICc  | Weight |
|--------------------|--------------------------|----------|----------------|--------|--------|--------|
| Body Mass (adults) | SVL+Altitude             | 5        | -62.162        | 134.7  | 0.00   | 0.352  |
|                    | SVL+Altitude+Water       | 6        | -61.576        | 135.7  | 0.99   | 0.215  |
|                    | SVL+Altitude+Forest      | 6        | -62.162        | 136.9  | 2.16   | 0.120  |
|                    | SVL                      | 4        | -65.039        | 138.3  | 3.62   | 0.058  |
|                    | SVL+Temperature          | 5        | -64.413        | 139.2  | 4.50   | 0.037  |
|                    | SVL+Q                    | 5        | -64.533        | 139.5  | 4.74   | 0.033  |
|                    | SVL+Forest               | 5        | -64.602        | 139.6  | 4.88   | 0.031  |
|                    | SVL+Water                | 5        | -64.642        | 139.7  | 4.96   | 0.029  |
|                    | SVL+Precipitation        | 5        | -64.834        | 140.1  | 5.34   | 0.024  |
|                    | SVL+Temperature+Water    | 6        | -64.023        | 140.6  | 5.88   | 0.019  |
|                    | SVL+Temperature+Forest   | 6        | -64.170        | 140.9  | 6.18   | 0.016  |
|                    | SVL+Q+Water              | 6        | -64.202        | 141.0  | 6.24   | 0.016  |
|                    | SVL+Q+Forest             | 6        | -64.285        | 141.1  | 6.41   | 0.014  |
|                    | SVL+Water+Forest         | 6        | -64.337        | 141.2  | 6.51   | 0.014  |
|                    | SVL+Precipitation+Water  | 6        | -64.454        | 141.5  | 6.74   | 0.012  |
|                    | SVL+Precipitation+Forest | 6        | -64.529        | 141.6  | 6.89   | 0.011  |
|                    | Precipitation            | 4        | -120.190       | 248.6  | 113.92 | 0.000  |
|                    | Altitude                 | 4        | -120.533       | 249.3  | 114.61 | 0.000  |
|                    | Q                        | 4        | -121.297       | 250.9  | 116.14 | 0.000  |
|                    | Temperature              | 4        | -122.318       | 252.9  | 118.18 | 0.000  |
| Forest             | 4                        | -124.126 | 256.5          | 121.80 | 0.000  |        |
| Water              | 4                        | -124.748 | 257.8          | 123.04 | 0.000  |        |

(conditional average)

|                      | Estimate   | Std. Error | Adjusted SE | z value | Pr(> z ) |     |
|----------------------|------------|------------|-------------|---------|----------|-----|
| <i>(Intercept)</i>   | -4.3055250 | 0.5828518  | 0.5898320   | 7.300   | <2e-16   | *** |
| <i>SVL</i>           | 0.1375678  | 0.0104824  | 0.0106080   | 12.968  | <2e-16   | *** |
| <i>Altitude</i>      | 0.0002489  | 0.0001014  | 0.0001037   | 2.401   | 0.0163   | *   |
| <i>Water</i>         | -0.0454223 | 0.0454784  | 0.0464918   | 0.977   | 0.3286   |     |
| <i>Forest</i>        | 0.0145925  | 0.0498932  | 0.0508558   | 0.287   | 0.7742   |     |
| <i>Temperature</i>   | -0.0252600 | 0.0236610  | 0.0241857   | 1.044   | 0.2963   |     |
| <i>Q</i>             | 0.0005441  | 0.0005834  | 0.0005963   | 0.912   | 0.3615   |     |
| <i>Precipitation</i> | 0.0002529  | 0.0004485  | 0.0004584   | 0.552   | 0.5812   |     |



Partie II : Lien entre la balance hydrique et l'alimentation chez un mésoprédateur ectotherme terrestre



## Résumé :

Cette partie porte sur les liens entre balance hydrique et alimentation à l'échelle individuelle, chez le modèle d'étude, *Zootoca vivipara*, étudié ici en tant que prédateur. Elle comprend deux chapitres qui sont actuellement soumis pour publication.

Le premier chapitre provient d'un travail principalement réalisé par Théo Bodineau lors de son stage de Master 2 ayant eu lieu pendant la thèse. Dans cette étude comportementale, les stratégies de thermo-hydrorégulation mises en place en réponse à une sécheresse sont étudiées. Les effets de la disponibilité alimentaire et de l'humidité du microhabitat sur ces réponses sont expérimentalement testés par la manipulation de l'accès à la nourriture et de la qualité hydrique d'un abri. La restriction hydrique provoque des altérations morphologiques et physiologiques telles que le catabolisme musculaire et la mobilisation de réserves caudales, mais entraîne également des stratégies comportementales pour conserver l'eau comme la diminution du temps passé à thermoréguler et l'augmentation de l'utilisation de l'abri. Ces changements sont principalement atténués par la présence d'un abri humide, mais pas par l'accès à de la nourriture. En effet, l'apport alimentaire n'a pas joué ici de rôle majeur dans la régulation de l'état d'hydratation et a accru les conflits comportementaux entre la thermorégulation et l'hydrorégulation. Ces résultats soulignent l'importance de prendre en compte les interactions complexes entre l'alimentation et la balance hydrique pour comprendre les réponses des ectothermes à des variations environnementales.

Le deuxième chapitre présente une étude écophysiological permettant de tester expérimentalement si la nourriture permet de rétablir la balance hydrique chez un lézard insectivore. Pour cela, des lézards vivipares adultes sont placés en condition de déshydratation modérée, puis nourris avec différents types de proies invertébrées issues de leur régime alimentaire naturel. Des mesures physiologiques sont utilisées pour évaluer leur état d'hydratation et suivre la réponse globale de l'organisme à la déshydratation. Nos résultats indiquent que les lézards vivipares ne peuvent pas améliorer leur état d'hydratation par la consommation de proies, quel que soit le type de proie, suggérant qu'ils dépendent principalement de l'eau de boisson. Cependant, la consommation de proies de bonne qualité réduit les coûts énergétiques liés à la privation d'eau, ce qui peut aider les lézards à conserver une meilleure condition corporelle pendant les périodes de sécheresse. Ces résultats permettent de mieux comprendre les réponses physiologiques des ectothermes au stress hydrique et mettent en évidence de probables compromis entre maintien de la balance hydrique et de la balance énergétique chez les lézards insectivores.

## **Chapitre 3) Microhabitat humidity rather than food availability drives thermo-hydroregulation responses to drought in a terrestrial lizard**

*Submitted for publication in Oikos*

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Running title: Effects of microhabitat quality and food availability on thermo-hydroregulatory strategies of a dry-skinned ectotherm

Acknowledgement:

The authors thank Damien Lopez Santin and Anouk Pellerin who contributed to behavioural and physiological data acquisition. This work was supported by an Agence Nationale de la Recherche grant (ANR-17-CE020013, 'AQUATHERM') to J.-F. Le Galliard. Théo Bodineau's PhD grant is funded by the Ministère de l'Enseignement Supérieur et de la Recherche. This work has benefited from human and technical resources provided by the CEREPEE-Ecotron IleDeFrance (CNRS/ENS UMS 3194).

Authors' contributions:

Théo Bodineau, Jean-François Le Galliard, Chloé Chabaud and Olivier Lourdais conceived the ideas and designed methodology; Théo Bodineau, Chloé Chabaud, Simon Agostini, Beatriz Decencièrè, Sandrine Meylan and Jean-François Le Galliard participated to lizard captures and breeding; Théo Bodineau and Jean-François Le Galliard collected and analysed the data; Théo Bodineau led the writing of the manuscript with Jean-François Le Galliard. All authors contributed critically to the drafts and gave final approval for publication.

Conflict of Interest:

The authors declare no competing or financial interests.

Data accessibility:

Data will be deposited in Zenodo after acceptance.

## Abstract

1. The regulation of energy balance, water balance and body temperature involve integrated processes that underlie some of the ecological responses of ectotherms to climate change. Functional trade-offs between thermoregulation and hydroregulation are exacerbated during hot or dry spells, but how microhabitat hydric properties and trophic resource availability influence these trade-offs remains unknown.

2. Here, we investigated the effects of microhabitat humidity and food availability on thermo-hydroregulation strategies in the ground-dwelling common lizard (*Zootoca vivipara*) during a simulated hot and dry spell event. We exposed lizards to a five-day long acute water restriction in hot conditions in the laboratory and manipulated hydric quality of the retreat site (wet or dry shelter) as well as food availability (*ad libitum* food or food deprivation).

3. Water restriction and food deprivation caused physiological alterations such as muscle catabolism and mobilization of caudal energy reserves. Lizards also developed behavioural strategies to conserve water or energy via decreased thermoregulation effort, higher shelter use and increased eye closure behaviours through time. Plasma osmolality (dehydration) increased on average during the experiment.

4. These physiological and behavioural changes were importantly buffered by the presence of a wet shelter but not by food availability. A wet retreat site reduced the behavioural conflicts between thermoregulation and hydroregulation, allowed lizards to maintain a better condition and reduced physiological dehydration. Instead, food intake did not play a major role in the regulation of hydration state and increased behavioural conflicts between thermoregulation and hydroregulation.

5. A better consideration of thermo-hydroregulation behaviours and microhabitat hydric quality is required to address ectotherm responses to future climate change.

Keywords: behaviour, food, microhabitat, shelter, thermo-hydroregulation, trade-off, water

## Introduction

The vulnerability of ectotherms to global warming is now well documented and involves lethal effects of high temperatures as well as sub-lethal effects due to energy imbalance, dehydration risks, or chronic stress responses (Burraco et al., 2020; Jørgensen et al., 2022; Kingsolver et al., 2013; Whitfield et al., 2007). Behavioural thermoregulation, which encompasses a range of behavioural processes involved in the regulation of body temperature (Angilletta Jr., 2009), is a critical determinant of the sensitivity of ectotherms to these acute and chronic effects of climate warming (Gunderson & Leal, 2015; Kearney, 2013; Sinervo et al., 2010). Indeed, terrestrial ectotherms can shift their activity patterns and microhabitat use in response to heat stress, often searching for cooler microhabitats during hot weather episodes (Beck & Jennings, 2003; Ryan et al., 2016), with net consequences for their fitness and population dynamics (Kearney, 2013). However, recent empirical studies and mechanistic models have both highlighted that the behavioural capacity of ectotherms to buffer consequences of climate warming may be constrained by water availability in the environment, which may further exacerbate the deleterious effects of heat stress (Kearney et al., 2018; Le Galliard et al., 2021; Lorenzon et al., 1999; Rozen-Rechels et al., 2020; Sannolo & Carretero, 2019). For example, water restriction may reduce activity periods during heat stress and compromise even more energy acquisition (e.g. Davis & DeNardo, 2009).

That thermoregulation is intertwined with water balance regulation (i.e., hydroregulation) has led to the concept of thermo-hydroregulation strategies, defined as suites of functionally integrated physiological and behavioural processes involved in the joint regulation of body temperature and water balance (Rozen-Rechels et al., 2019). A proximate conflict exists between regulation of water balance and thermoregulatory behaviours such as basking and maintenance of high body temperatures, since those typically increase respiratory and cutaneous water loss rates and eventually lead to dehydration and decreased performances (Anderson & Andrade, 2017; Dmi'El, 2001; Preest & Pough, 1989). Conversely, dehydration state can constrain thermal preference to promote water conservation (Ladyman & Bradshaw, 2003; Le Galliard et al., 2021; Rozen-Rechels et al., 2020; Sannolo & Carretero, 2019). This functional trade-off has consequences for behavioural patterns of microhabitat selection: for example, excessively hot and dry weather events may force terrestrial ectotherms to select cooler and wetter microhabitats (i.e., shaded areas or underground shelters) that are less desiccating, i.e., favourable for hydroregulation, but suboptimal for thermoregulation (Pintor et al., 2016; Rozen-Rechels et al., 2020). Currently, besides recent studies focusing on behavioural

consequences of water availability (Rozen-Rechels et al., 2020; Sannolo & Carretero, 2019), we have limited knowledge of environmental factors that influence behavioural trade-offs between thermoregulation and hydroregulation and may act as environmental buffers against the dual deleterious effects of heat and water stress (Dezetter et al., 2022).

Food resources should influence thermo-hydroregulation strategies because those should be constrained by energy intake according to the cost-benefit model of thermoregulation (Angilletta Jr., 2009; Huey & Slatkin, 1976). In heliothermic ectotherms, thermoregulatory behaviours involve shuttling between different microclimates, which may increase activity and energy expenditure (Angilletta Jr., 2009). Thus, the thermoregulation effort and precision can be compromised when food availability is limited, although experimental studies have shown that these adjustments are quite variable among species (Brown & Griffin, 2005; Gilbert & Miles, 2016; Huey & Slatkin, 1976; Schuler et al., 2011). Food could also influence hydroregulation behaviours if it allows to recoup some of the energetic costs of hydroregulation and because food can provide dietary water, a scenario which we called the "food for water hypothesis". This hypothesis has been little tested so far in terrestrial ectotherms and we do not know whether prey consumption can meet water requirements when water resources become limited (Lillywhite, 2017). Several desert species of herbivorous, carnivorous, or insectivorous reptiles depend on dietary water to maintain their hydration status (Green et al., 1991; Nagy et al., 1991). In contrast, in several snake species and in one carnivorous lizard species, feeding was ineffective in maintaining hydration status and even increased reliance on drinking water that is essential during digestion, excretion of nitrogenous metabolic waste, and defecation (Lillywhite, 2017; Murphy & DeNardo, 2019; Wright et al., 2013). To our knowledge, only one study has investigated the interactive effects of food intake and hydration status on thermoregulatory behaviours (Perez et al., 2021). In the heliothermic tropical lizard *Tropidurus catalanensis*, food intake was the strongest predictor of preferred body temperatures, but neither hydration status alone or its interaction with food intake had significant effects on thermal preferences.

In addition, the availability and overall quality of microhabitats should importantly influence behavioural trade-offs between thermoregulation and hydroregulation. Heterogeneous microhabitats provide ectotherms with contrasted microclimatic conditions, lower the costs of behavioural thermoregulation and reduce the deleterious consequences of climate warming (Potter et al., 2013; Scheffers et al., 2014; Woods et al., 2015). In terrestrial ectotherms, wet and cold microhabitats provide thermal shelters to escape extreme surface



temperatures but also reduce evaporative water loss and dehydration stress (Beck & Jennings, 2003; Dezetter et al., 2022; Green, 1972; Moore et al., 2018). In dry-skinned ectotherms such as reptiles, hydroregulatory behaviour through selection of moist microhabitats independent from thermoregulation was just recently demonstrated experimentally (Dezetter et al., 2022; Pintor et al., 2016). These studies uncovered that elevated ambient temperatures promote retreat behaviours in a moist shelter independently from the temperature of the shelter. Thus, in heliothermic ectotherms, heterogeneous habitats including high hydric quality shelters should mitigate the impact of water constraints and influence thermo-hydroregulation strategies compared to more homogeneous habitats. Nevertheless, to our knowledge, no study has quantified the effects of microhabitat quality on thermo-hydroregulation strategies and on fitness consequences of a hot and dry weather event.

Here, we designed an experiment in order to quantify the effects of food availability and hydric microhabitat quality on thermo-hydroregulation strategies and fitness proxies during a hot and dry weather event simulated in the laboratory in the common lizard *Zootoca vivipara* (Lichenstein, 1823). This small diurnal, insectivorous lacertid, is found in cool and moist environments across northern Eurasia, is an heliothermic lizard with an active thermoregulation strategy and is sensitive to water restriction due to its high standard cutaneous water loss rates (Herczeg et al., 2003; Lorenzon et al., 1999; Rozen-Rechels et al., 2020). We removed access to free-standing water for several days to simulate an extreme summer drought with strong water restriction. We further manipulated food availability (*ad libitum* food vs. no food) as well as the quality of a surface shelter (access to a wet cold shelter or to a dry cold shelter), which is generally used by lizards for rest during daytime and night-time inactivity phases. In the same time, we quantified the behavioural adjustments of the lizards as well as their hydric and energetic conditions. We examined the following four hypotheses and predictions. First, water and food restriction should challenge homeostasis and compromise the energy and water balance of lizards. We predict a decrease in body condition in response to water restriction and food deprivation and an increase in plasma osmolality in response to water restriction. Second, compensatory physiological mechanisms should occur under conditions of water and food restriction. We predict muscle catabolism and mobilisation of caudal lipid reserves to release amino acids, lipids, and bound water and recoup some of the missing inputs of food and water (Brusch et al., 2018, 2020, 2022). Third, behavioural responses should mitigate water and energy constraints: we predict a decrease in selected body temperatures and thermoregulation effort, an increase in shelter use and more frequent water conservation strategies such as eye

closure behaviours in response to water and food restriction to promote water and energy conservation. Fourth, the behavioural trade-off between thermoregulation and hydroregulation should be stronger when shelters are sub-optimal (i.e., dry microclimatic conditions) and when food is deprived. In addition, deleterious effects of water restriction on body condition should be reduced by the presence of a wet shelter and food availability if food provides a net dietary water intake.

## **Material and methods**

### **Study species and sampling**

European common lizards (*Zootoca vivipara*) used in this study were captured inside semi-natural populations maintained in outdoor enclosures at CEREEP-Ecotron IleDeFrance (48°17'11.5 "N, 2°40'45.6 "E). These enclosures include natural vegetation with shelters and basking sites made of rocks and logs. In the enclosures, food includes natural prey such as small invertebrates (spiders, worms, insects) and lizards have permanent access to free standing water. Between April 21<sup>st</sup> and May 24<sup>th</sup> 2021, we captured 60 adult males aged two years or more (snout to vent length, SVL:  $58.9 \pm 2.17$  mm; body mass:  $4.32 \pm 0.50$  g) in 3 sequential capture sessions to form 3 sequential batches of 20 lizards. Prior to experiments, we acclimated lizards in individual terraria (18 × 11 × 12 cm) filled with sterilised peat as substrate, equipped with a shelter, and installed in a temperature-controlled room (23°C from 9 am to 6 pm, 16°C at night). In order to standardise habituation to laboratory conditions and food satiety, all lizards were acclimated in the laboratory for a minimum of 5 nights and 5 days prior to the experiments by providing food every second day (200 ± 10 mg of domestic crickets *Acheta domestica*), water *ad libitum* in a Petri dish and 3 water sprays per day (at 9 am, 12 pm and 5 pm). Experiments were performed under the approval of Charles Darwin Animal Experimentation Ethics Committee (APAFIS#30217-2021030322398713) in an animal breeding facility accredited with permit 17/DDPP/SPAE/57.

### **Experimental conditions and study design**

The experiment was performed in open-top experimental arenas (N=20, 78 × 56 × 44 cm) installed in a temperature-controlled room (23°C from 9 am to 6 pm, 16°C at night). Arenas were lit automatically between 8:30 am and 6 pm by a UVB-enhanced neon tube (30 W) providing white light and by three halogen heating bulbs (two 40W bulbs and one 50W) placed on one side of the terrarium in order to generate a thermal gradient (operative temperatures

from hot to cold spot:  $35.8\text{ }^{\circ}\text{C} \pm 7.5\text{ SD}$  to  $22.1\text{ }^{\circ}\text{C} \pm 2.5\text{ SD}$ ; see Figure S1 of Supplementary Information). This thermal gradient is relevant to the thermal biology of the common lizard because it encompasses a range from inactivity to optimal preferred temperatures (Gvoždík & Castilla, 2001).

Each arena was filled with 3 cm of peat and equipped with three equidistant water-filled Petri dishes placed in the middle and at each end of the arena to provide lizards with free-standing water. In the cold side of the arena, we installed a “shelter” made from a plastic box painted black with a 3 cm diameter entrance hole and a plastic mesh floor to elevate the lizard above a sandy substrate. The sandy substrate, inaccessible to the lizard, was moistened or not to create a wet (RH:  $95.92\% \pm 6.35\text{ SD}$ ) or a dry microhabitat (RH:  $50.50\% \pm 10.37\text{ SD}$ , see Figure S2 of Supplementary Information). The shelter, placed on the cold side of the gradient, provided lizards with a cold refuge and its average temperature did not differ significantly between dry and wet treatments (see Figure S2 of Supplementary Information). These custom-made standardized shelters thus mimic the cold refuges used by reptiles during their diurnal or nocturnal phases of inactivity (Beck & Jennings, 2003). Shelters were thermally sub-optimal for basking but had optimal hydric conditions or not depending on treatment conditions in our study. Note that we did not observe water condensation inside the shelters and lizards could not extract free standing water from the wet shelters.

We manipulated both food availability (food treatments: *ad libitum* feeding with  $200 \pm 20$  mg of crickets per day for the control treatment or no feeding) and humidity of the shelters (shelter treatments: access to wet shelter for the control treatment or access to a dry shelter) in a balanced full-factorial experimental design. Each batch of 20 lizards was divided into four experimental groups formed by the factorial combination of the two treatments and lizards were randomly assigned to a treatment group and a given arena. The study was repeated three times independently with our three batches of lizards. During the course of the experiment (see Figure S3 of Supplementary Information), lizards spent seven successive days in the same arena including one day of acclimation (called “day -1”) when lizards were maintained under control conditions with water, *ad libitum* food ( $200 \pm 20$  mg crickets), wet shelter, and three water sprays per day (at 8 am, 12 pm, and 5 pm). During “day 0”, we observed lizards under the same control conditions as during the acclimation day. At the end of this first day of observation, we removed water cups from all arenas, removed crickets for the food-deprived arenas, and replaced wet shelters with dry shelters in treatment groups with access to a dry shelter. From day 1 to day 5, a limited amount of water was provided to the lizards at 8:30 am by spraying

them with water for five seconds. This manipulation was done to generate a chronic, sub-lethal water restriction similar to exposure to several days of warm and dry weather in natural conditions (Dupoué et al., 2020). After the last behavioural observation on day 5, all individuals were provided with water *ad libitum* and transferred to standard housing conditions with or without food depending on their treatment group.

In two additional arenas without lizard, we measured the thermal characteristics of the warm and cold sides of the arena using operative temperature copper models mimicking the lizards thermal properties and equipped with HOBO® Pro v2 U23-003 (ONSET 188 Cape Cod, Massachusetts) temperature loggers (see details in Rozen-Rechels et al., 2020). We also measured air humidity and temperature by placing two iButtons (DS1922L-F5, Maxim Integrated Products, San Jose, CA, USA) in each shelter. These data are provided as Supplementary Information.

### **Pre- and post-experimental measurements**

Measurements were taken to quantify morphological and physiological changes during the experiment. At the beginning and at the end of the experiment (respectively on the evening of day -1 and at the end of day 5), lizards were bled using a standard protocol within 3 minutes of restraining the animal to avoid handling-induced stress effects on measurements (Bonnet et al., 2020; Meylan et al., 2003). Approximately 40  $\mu$ L of blood was collected from the post-orbital sinus and centrifuged for six minutes at 11,000 rpm. Plasma was then separated from red blood cells, aliquoted to allow subsequent measurements, and kept at  $-25^{\circ}\text{C}$  in Eppendorf tubes until analyses were performed.

Plasma osmolality, an indicator of hydration status in species lacking a salt gland (Peterson, 2002), was measured from two aliquots of plasma diluted in physiological serum using a Vapro® vapour pressure osmometer (model 5600; Wescor Inc., Logan, UT, USA; intra-assay repeatability:  $r = 0.94$ ,  $F_{117,118}=34.05$ ,  $p < 0.0001$ ). Osmolality was calculated as the average osmolality of the two aliquots corrected by dilution with the osmolality of a reference physiological serum ( $288.9 \text{ mOsm.kg}^{-1} \pm 3.6 \text{ SD}$ ). Circulating triglyceride and glucose levels were measured in plasma by colorimetric assays using 2.5 $\mu$ l and 1.5 $\mu$ l of plasma, respectively (Triglyceride Colorimetric Assay kit and Glucose Colorimetric Assay kit, ref. 10010303 and 10009582, Cayman Chemical, USA). This method quantifies the total triglycerides and glucose concentration by using a chain of enzymatic reactions ending in pink dye quantified by absorbance at 540 nm and 500 nm, respectively (iMark MicroPlate Reader, Bio-Rad, USA).

Samples were run together with a calibration standard and some pooled samples (four intra-plate and six inter-plate pools per assay) in two assays and we analysed repeated samples of the same individuals on the same assay. Measurements were highly repeatable (triglycerides intra-assay repeatability:  $r = 0.85$ ,  $F_{7,8}=12.45$ ,  $p < 0.001$ ; triglycerides inter-assay repeatability:  $r = 0.90$ ,  $F_{3,4}=19.32$ ,  $p = 0.008$ ; glucose intra-assay repeatability:  $r = 0.85$ ,  $F_{18,19}=12.98$ ,  $p < 0.0001$ ; glucose inter-assay repeatability:  $r = 0.83$ ,  $F_{19,20}=10.64$ ,  $p < 0.0001$ ).

In addition to this, lizards were weighed before the experiment (day -2), then every two days after the last behavioural record of the day (day 0, day 2, day 4) and finally at the end of the experiment (day 5) using a precision scale (PX 323 Pioneer, OHAUS Corp., USA, accuracy: 1 mg). At the end of day 6, we weighed each lizard to measure post-manipulation water intake and feeding behaviours. Water intake and feeding was inferred from the body mass changes between day 5 to day 6 (Dupoué et al., 2014; Lillywhite et al., 2008). Before placing the individuals in the arenas (day -2) and at the end of the experiment (day 5), we also scanned the lizards ventrally using a flatbed scanner (CanoScan LiDE 700F, Canon) to measure tail width, as a proxy for tail reserves, at the 7<sup>th</sup>, 9<sup>th</sup>, and 12<sup>th</sup> sub-caudal scale rows using ImageJ software (Version 1.52v). At the same time, hind limb thickness was measured on both legs using a spessimeter (ID-C1012BS, Mitutoyo, Japan, accuracy: 0.01 mm).

### **Behavioural measurements**

We recorded body temperature and behaviours of lizards for 6 days (day 0 to day 5; see Figure S3 of Supplementary Information) with a focal sampling every 30 minutes from 9 am to 5 pm (i.e., 17 readings during the day) where we noted the position (inside or outside the shelter) and the behaviour (basking, motionless, feeding, mobile, fleeing, or buried) of each lizard. To avoid disturbance of lizards, surface body temperature (called  $T_{IR}$ ) was recorded at a distance of 60 cm using an infrared (IR) thermometer (Raytek, Raynger MX2). This provided us with a non-invasive measure that is highly correlated with core body temperature (Artacho et al., 2013). Indeed, cloacal temperatures (called  $T_b$ ) measured with a K-type thermocouple (HI 935005N, Hanna,  $\pm 0.1^\circ\text{C}$ ) after the last behavioural record of day 0 were strongly correlated with surface IR temperature ( $N=60$ ,  $T_b = 7.46 (\pm 0.90) + 0.70 (\pm 0.03) \times T_{IR}$ ,  $R^2 = 0.91$ , see Figure S4 of Supplementary Information). When the lizard was not buried or in the shelter, we noted whether its eyes were open or closed. When the lizard was not visible, the roof of the shelter was slowly lifted to note the presence or not of the lizard in the shelter. In addition, we recorded whether or not the animal was in the shelter outside of the activity phases at 9 pm and 7 am between day

-1 and day 5. Since 96% of the records were similar at 9 pm and 7 am, we used only data from observations at 7 am

### **Statistical analyses**

Analyses were performed with R software version 4.2.0 (R Core Team, 2022). For each analysis, we built a full model, checked its assumptions and performed a goodness-of-fit test in some cases. Non-significant effects were then eliminated using a stepwise model selection procedure starting from the full model. Stepwise model selection was based on standard  $F$  statistics for linear models and likelihood ratio chi-square test for generalised linear models using the Anova procedure of *car* package (Fox & Weisberg, 2019). First, we calculated intra-individual changes in body mass (from day -2 to day 5 and independently after rehydration from day 5 to day 6), in limb thickness, in tail width, in plasma osmolality, in triglyceride and in glucose concentration by subtracting the final values measured after the experiment from the initial values measured before the experiment. For the change in tail width, we calculated this change at the 7th, 9th and 12th row of sub-caudal scales. For the change in limb thickness, we calculated this change at the right and left hind legs and then averaged them. We fitted linear mixed models with the *nlme* package (Pinheiro et al., 2012) to explain these intra-individual changes by the effects of fixed factors including experimental batch (class with 3 levels, corresponding to the 3 sequential batches of each experiment), the initial centred value of the response variable and interactive effects of the two experimental treatments (shelter and food treatment). For the analysis of the change in plasma osmolality, we calculated an individual-centred shelter use score during the experiment and added this variable in interaction with the shelter and food treatment because we wanted to test the relationship between shelter use and dehydration (Dezetter et al., 2022). For tail width analysis, a fixed factor corresponding to the 3 rows of scales where tail width was measured (class with 3 levels) was added to the model. In addition, we included a random intercept term to account for potential differences among experimental arenas. For changes in body mass and plasma osmolality that respond very rapidly to changes in water availability (Shoemaker & Nagy, 1977), we removed two individuals from the analyses that were sprayed with water for 5 seconds on day 4 in accordance with the human endpoints of the protocol. One lizard was also removed from the analyses for triglycerides and two for glucose because of insufficient plasma quantity to perform the assays.

Body temperature  $T_b$  was calculated from surface IR temperatures using the calibration curve (see Supplementary Information S4) and was analysed using linear mixed models

including fixed effect of experimental batch, factorial or linear effects of time (number of days since start of the experiment), factorial effects of experimental treatments, and the interaction between time and treatments. A second analysis of hourly  $T_b$  variation was performed using linear mixed models including a four-way interaction between the quadratic or linear effect of daytime (hour of the day), the linear or factorial effect of time (number days), shelter treatment and food treatment. The random part of these models included two nested, uncorrelated components: a random intercept term to control for differences among individuals within the same arena and a random intercept term to control for differences among arenas. To ensure a Gaussian distribution of the residuals, we performed a Box-Cox transformation of raw data.

To study thermo-hydroregulation behaviours, we counted the number of times each day out of 17 observations where lizards exhibited basking behaviour, when they closed their eyes, and when they were in the shelter (at 7 am, see above). We then analysed these counts data as success-failure events with generalised linear mixed models assuming a binomial distribution and a logit link using the *glmer* function from the *lme4* package (Bates et al., 2014). These models had a satisfactory fit to the data based on Pearson's chi-square goodness-of-fit tests and examination of residuals. For basking and eye closure behaviours, we fitted the models with the experimental batch and the factorial or linear effects of time (number of days since start of the experiment) in interaction with the experimental treatments. For daytime and nighttime shelter use behaviours, the models were fitted with the experimental batch and the factorial, linear or quadratic effect of time in interaction with the experimental treatments. We also analysed hourly basking behaviours variation using generalised linear mixed models including a four-way interaction between the quadratic or linear effect of daytime (hour of the day), the linear or factorial effect of time, shelter treatment and food treatment. In all cases, we controlled for inter-individual variability by including a random effect of individual identity.

## **Results**

### **Shelter use**

Daytime shelter use was significantly influenced by the interaction between time (number of days since start of the manipulation), shelter treatment and food treatment (time  $\times$  shelter treatment  $\times$  food treatment:  $\chi^2_1 = 8.68$ ,  $p = 0.003$ ). As the number of days of water restriction increased, average daytime shelter use increased in most lizards (see Figure 1A). In food-deprived animals, daytime shelter use increased similarly in dry and wet shelter treatment groups, whereas in fed lizards, daytime shelter use increased more strongly in lizards provided

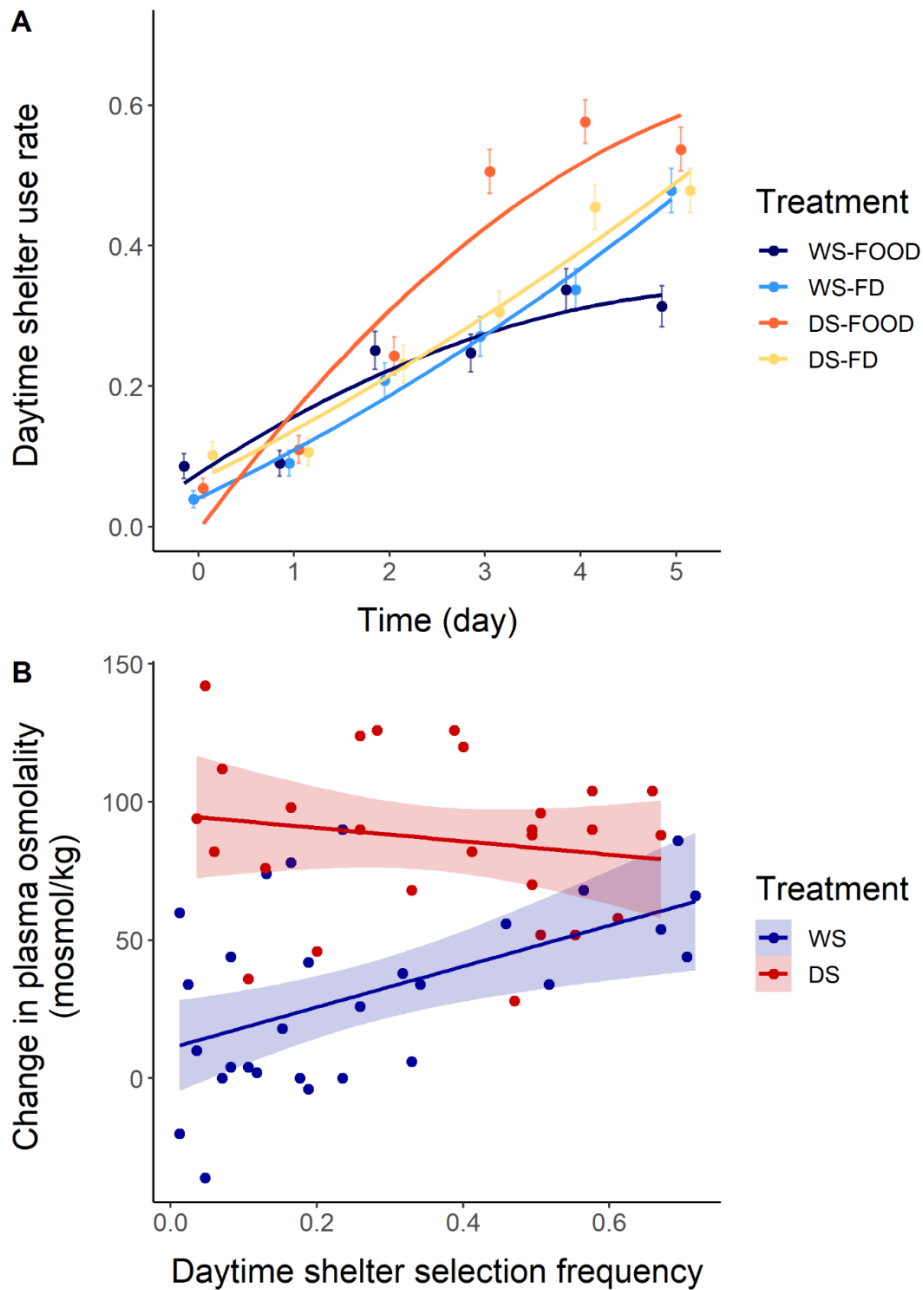
with a dry shelter than with a wet shelter. Instead, nighttime shelter use increased over time regardless of treatments (time:  $\chi^2_1 = 25.26$ ,  $p < 0.0001$ ; shelter treatment:  $\chi^2_1 = 1.62$ ,  $p = 0.20$ ; food treatment:  $\chi^2_1 = 1.61$ ,  $p = 0.20$ ; see Figure S5 of Supplementary Information).

### **Body temperature and thermo-hydroregulation behaviours**

Average  $T_b$  of the day was influenced by the shelter treatment in interaction with time but not by the food treatment (time  $\times$  shelter treatment:  $F_{1,2514} = 11.04$ ,  $p < 0.0001$ , time  $\times$  food treatment:  $F_{1,2509} = 1.45$ ,  $p = 0.20$ ). In general, the average daily  $T_b$  of lizards decreased through time during the experiment. This decrease was more pronounced in lizards provided with dry shelter ( $-2.7$  °C in five days) compared to lizards with a wet shelter ( $-0.7$  °C in five days; see Figure 2A, B). In addition, hourly patterns of  $T_b$  were influenced by a complex, four-way interaction between time, daytime (hour of the day), shelter and food treatments (time  $\times$  hour  $\times$  shelter treatment  $\times$  food treatment:  $F_{5,2462} = 3.17$ ,  $p = 0.007$ ). In control condition (day 0),  $T_b$  increased in the morning and decreased slightly in the afternoon (see Figure S6 of Supplementary Information). Dry shelters and food deprivation caused respectively a lower  $T_b$  selection at the beginning and at the end of the day and a decrease in  $T_b$  in the afternoon. Nevertheless, the decrease in afternoon  $T_b$  in response to food deprivation was exacerbated in lizards with dry shelter.

Basking behaviour was significantly influenced by the interaction between time and the shelter treatment but not by the food treatment (time  $\times$  shelter treatment:  $\chi^2_5 = 45.35$ ,  $p < 0.001$ ; food treatment:  $\chi^2_1 = 0.09$ ,  $p = 0.77$ ). The proportion of basking behaviours decreased smoothly during the experiment, with a more pronounced decrease in lizards from the dry shelter treatment especially at the start of the experiment (see Figure 2C, D). Hourly patterns of basking behaviour were influenced by an interaction between a quadratic effect of time, daytime and shelter treatment irrespective of food (time  $\times$  hour  $\times$  shelter treatment:  $\chi^2_5 = 15.64$ ,  $p = 0.008$ ; food treatment:  $\chi^2_1 = 0.12$ ,  $p = 0.73$ ). Under control conditions, basking behaviours were maximal in the morning and slowly decreased in the afternoon (see Figure S7 of Supplementary Information). Then, we observed a gradual shift in basking behaviours towards the earlier hours of the day during the manipulation, which was stronger in the presence of a dry shelter. Finally, we found strong effects of shelter treatment in interaction with time on the ocular behaviour of lizards (time  $\times$  shelter treatment:  $\chi^2_5 = 17.05$ ,  $p < 0.001$ ). Eye closure rate increased remarkably more in lizards provided with a dry shelter compared to lizards with a wet shelter (see Figure 2E, F).





**Figure 1. A.** Interactive effects of shelter and food treatment on daytime shelter use as a function of time (number of days since the start of the experiment). **B.** Plasma osmolality change during the experiment as a function of frequency of shelter use and shelter treatment. Colours in panel A represent different experimental groups formed by the factorial combination of the two treatments (WS-FOOD (dark blue): wet shelter and *ad libitum* feeding, WS-FD (light blue): wet shelter and food deprivation, DS-FOOD (orange): dry shelter and *ad libitum* feeding, DS-FD (yellow): dry shelter and food deprivation). Colours in (B) represent lizards with access to wet shelter (WS (dark blue)) or dry shelter (DS (red)). In (A), points represent average daily shelter use ( $\pm$  SE) and solid lines represent the average shelter use adjusted with a quadratic time effect. In (B), points represent individual shelter use score during the experiment and a positive change in osmolality indicates physiological dehydration. Water restriction begins on day 1.

| Treatment groups                         | Wet shelter               |                            | Dry shelter                |                           |
|--|---------------------------|----------------------------|----------------------------|---------------------------|
|  | Food                      | Food deprivation           | Food                       | Food deprivation          |
| <b>Variables</b>                         |                           |                            |                            |                           |
| Δ body mass (g)                          | -0.22 ± 0.05 <sub>a</sub> | -0.65 ± 0.05 <sup>b</sup>  | -0.67 ± 0.05 <sup>b</sup>  | -1.04 ± 0.05 <sup>c</sup> |
| Δ limb thickness (mm)                    | -0.07 ± 0.02 <sub>a</sub> | -0.14 ± 0.02 <sup>ab</sup> | -0.15 ± 0.02 <sup>bc</sup> | -0.21 ± 0.02 <sup>c</sup> |
| Δ tail width (mm)                        | -0.11 ± 0.10 <sub>a</sub> | -0.45 ± 0.10 <sup>b</sup>  | -0.52 ± 0.09 <sup>b</sup>  | -0.77 ± 0.10 <sup>c</sup> |
| Δ body mass during rehydration (g)       | 0.26 ± 0.26 <sup>a</sup>  |                            | 0.64 ± 0.18 <sup>b</sup>   |                           |
| Δ [triglycerides] (mg.dL <sup>-1</sup> ) | 256.4 ± 28.9 <sup>a</sup> | -57.2 ± 28.5 <sup>b</sup>  | 378.3 ± 29.7 <sup>c</sup>  | -47.8 ± 29.6 <sup>b</sup> |
| Δ [glucose] (mg.dL <sup>-1</sup> )       | 49.5 ± 11.0 <sub>ab</sub> | 24.1 ± 11.0 <sup>b</sup>   | 71.8 ± 11.0 <sup>a</sup>   | 8.22 ± 11.8 <sup>b</sup>  |

**Table 1.** Summary statistics of morphological and physiological changes in 60 males *Zootoca vivipara* from the four treatment groups (n = 15 per group). Morphological and physiological changes were calculated by subtracting the initial trait value measured at the beginning of the experiment from the final value measured at the end of the experiment. Δ limb thickness (mm) represents the average changes in tail width at the 7th, 9th, and 12th sub-caudal scale rows. Δ limb thickness (mm) represents the average of the left and right leg values. Data are provided as mean (± SE) for each treatment group and different letters indicate significant differences between groups (p < 0.05; Tukey's HSD post hoc test).

### Morphological changes

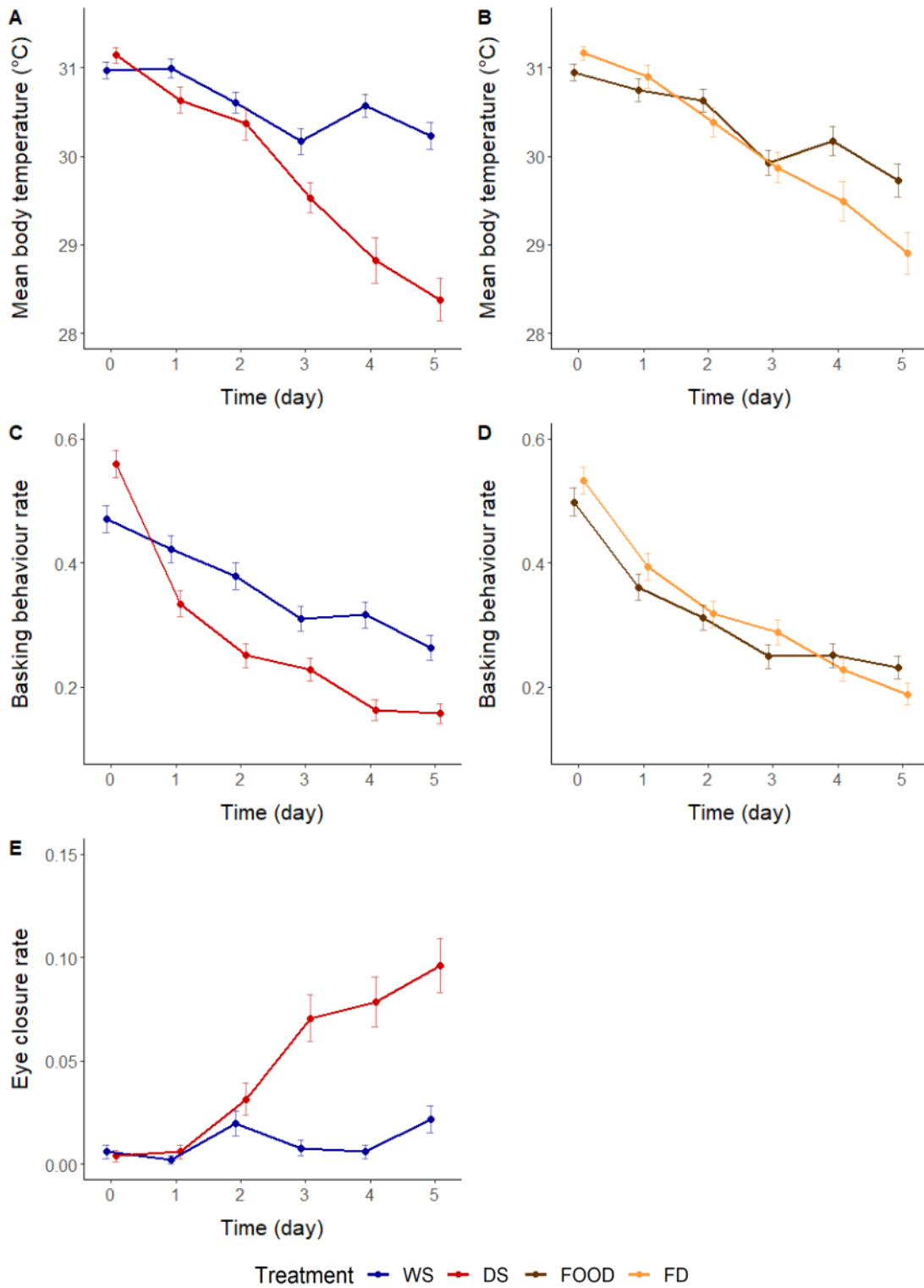
Lizards lost an average of  $0.30 \text{ g} \pm 0.06 \text{ SE}$  during the experiment, equivalent to 7.5 % of their initial body mass. Mass loss was additively influenced by shelter treatment and food treatment (shelter treatment:  $F_{1,33} = 82.95$ ,  $p < 0.0001$ ; food treatment:  $F_{1,33} = 69.41$ ,  $p < 0.0001$ ), but not by the interaction between these two treatments ( $F_{1,34} = 0.52$ ,  $p = 0.47$ ). Body mass loss was much greater for food-deprived lizards (contrast =  $-0.40 \text{ g} \pm 0.05 \text{ SE}$ ) and lizards with dry shelter (contrast =  $-0.43 \text{ g} \pm 0.05 \text{ SE}$ ; Table 1). Similarly mean limb thickness decreased on average by  $0.06 \text{ mm} \pm 0.02 \text{ SE}$  with additive but not interactive effects of food and shelter treatments (food treatment:  $F_{1,37} = 10.15$ ,  $p = 0.003$ ; shelter treatment:  $F_{1,37} = 18.15$ ,  $p = 0.0001$ ; food × shelter treatment:  $F_{1,36} = 0.09$ ,  $p = 0.77$ ; Table 1). Tail width decreased slightly and heterogeneously during the experiment by about  $-0.050 \text{ mm} \pm 0.11 \text{ SE}$  with additive but non-

interactive effects of shelter and food treatments (food treatment:  $F_{1,153} = 18.99$ ,  $p < 0.0001$ ; shelter treatment:  $F_{1,153} = 31.68$ ,  $p < 0.0001$ ; food treatment  $\times$  shelter treatment:  $F_{1,152} = 0.22$ ,  $p = 0.64$ ; Table 1). The increase in body mass during rehydration after the manipulation was only influenced by shelter treatment ( $F_{1,33} = 65.77$ ,  $p < 0.0001$ ; food treatment:  $F_{1,32} = 0.04$ ,  $p = 0.84$ ). Mass gain was greater for lizards who had a dry shelter prior to rehydration (Table 1).

### **Blood parameters changes**

We found an overall increase in plasma osmolality indicating strong physiological dehydration during the manipulation where water access was restricted ( $+57.09 \text{ mOsmol.kg}^{-1} \pm 5.6 \text{ SE}$ ). The change in plasma osmolality was influenced by the interaction between frequency of shelter use and shelter treatment independently of food treatment (frequency of shelter use  $\times$  shelter treatment:  $F_{1,30} = 4.44$ ,  $p = 0.04$ ; food treatment:  $F_{1,30} = 2.27$ ,  $p = 0.14$ ; Table 1). Dehydration was on average greater in lizards provided with a dry shelter ( $+86.74 \text{ mosmol.kg}^{-1} \pm 5.58 \text{ SE}$ ) compared to a wet shelter ( $+30.4 \text{ mosmol.kg}^{-1} \pm 6.01 \text{ SE}$ ) and it was correlated with daily shelter use but only in the group of lizards provided with a wet shelter (see Figure 1B).

Plasma glucose concentration was only influenced by food treatment (food treatment:  $F_{1,33} = 14.64$ ,  $p = 0.0005$ ; shelter treatment:  $F_{1,33} = 0.13$ ,  $p = 0.71$ ). Fed lizards showed a significant increase in plasma glucose concentration relative to food-deprived lizards (contrast =  $+43.46 \text{ mg.dL}^{-1} \pm 11.4 \text{ SE}$ ; Table 1). Finally, the change in plasma triglyceride concentration was influenced by the additive effects of shelter treatment and food treatment (food treatment:  $F_{1,33} = 152.5$ ,  $p < 0.0001$ ; shelter treatment:  $F_{1,33} = 4.61$ ,  $p = 0.04$ ), with the interaction between both factors being close to significance (food treatment  $\times$  shelter treatment:  $F_{1,32} = 3.70$ ,  $p = 0.06$ ). A greater increase in circulating plasma triglyceride concentration was observed in fed than food-deprived lizards (contrast =  $+368.25 \text{ mg.dL}^{-1} \pm 29.8 \text{ SE}$ ) and also to some extent in lizards from the dry shelter group than from the wet shelter group (contrast =  $+64.59 \text{ mg.dL}^{-1} \pm 30.1 \text{ SE}$ ; Table 1).



**Figure 2.** Effects of experimental treatments on thermo-hydroregulation behaviours of male *Zootoca vivipara*. Mean body temperature (A, B), mean basking rate (C, D), and eye closure rate (E) of lizards as a function of day and experimental treatment (WS: wet shelter, DS: dry shelter, FOOD: food *ad libitum*, FD: food deprivation). Points and error bars represent temperature, basking rate, and average daily eye closure rate of lizards ( $\pm$  SE). Water restriction begins on day 1.

## Discussion

We examined simultaneous effects of food availability and shelter humidity on thermo-hydroregulation behaviours, morphology, hydration status, and plasma metabolites in adult male lizards during a simulated chronic water restriction. Body mass decreased and dehydration increased on average during the manipulation, and there was a rapid gain of body mass following post-experiment access to free-standing water, which indicates that lizards became significantly dehydrated as we predicted (Dupoué et al., 2018; Rozen-Rechels et al., 2020). In addition, food-deprived lizards had lower circulating triglyceride concentrations and blood glucose levels, indicating that the food deprivation treatment induced fasting patterns consistent with previous studies on lipid or glucose metabolism in reptiles (McCue, 2007; Moon et al., 1999; Price, 2017). In parallel with the average degradation of the hydration status of lizards, water and food restriction resulted in a decrease in body mass, hind limb width, and tail width. In snakes and birds, muscle wasting is caused by protein catabolism and is associated with a significant release of protein-bound water to improve the water status during water restriction (Brusch et al., 2018; Gerson & Guglielmo, 2011). In lizards, including the common lizard, the tail is also a reserve organ consisting mostly of muscle and lipids stored as triglycerides (Avery, 1974; Price, 2017). Thus, our data suggest that fasting and dehydration caused mobilization of caudal lipid reserves and muscle wasting to support water, energy, and amino acid requirements when water and dietary intakes are insufficient (Cherel et al., 1992).

We found that lizards developed behavioural strategies likely to reduce evaporative water loss at the expense of thermoregulation effort and accuracy and more strongly in the dry shelter group. Observed behavioural responses were indicative of a functional trade-off between daily thermoregulation (i.e., lower basking effort and body temperatures through time) and hydroregulation (i.e., higher shelter use and eye closure through time) mediated by the hydric costs of thermoregulation (Le Galliard et al., 2021; Rozen-Rechels et al., 2020; Sannolo & Carretero, 2019). These behavioural changes were both immediate (i.e., from the first day of water restriction) and gradual, and are thus likely mediated by multiple cues such as the availability of water in the environment and the individual hydration status (Rozen-Rechels et al., 2020). In addition to these temporal trends, hydric quality of the shelter rather than food availability was the main modulator of the thermo-hydroregulation strategies and an important buffer against the deleterious effects of water restriction. Presence of a wet shelter at day and night limited very strongly physiological dehydration, muscle catabolism and mobilization of caudal lipid reserves. In addition, the absence of a wet shelter at day and night caused a much

stronger decrease through time in thermoregulation effort and a substantial reduction of the thermoregulation precision (i.e., lower body temperatures, reduced basking behaviours and shorter daily thermoregulation period), as predicted if stronger dehydration inflates the behavioural conflicts between thermoregulation and hydroregulation. Finally, absence of a wet shelter also caused a drastic increase in eye closure rate through time. Flexible adjustments of eye closure had already been observed in reptiles, especially when these animals are basking, and those are often interpreted as anti-predator adaptations (Lanham & Bull, 2004). Our study is the first experimental demonstration of an adjustment of eye closure behaviour in response to hydric stress. In dry-skinned ectotherms with an eyelid, eye closure behaviours are predicted to play an important role in organismal hydroregulation because the eye surface is poorly resistant to water loss and eye closure can contribute importantly to water balance regulation (Pirtle et al., 2019; Waldschmidt & Porter, 1987). Thus, our results imply that an under-appreciated trade-off will emerge between hydroregulation behaviours and vigilance in desiccating environments, with potential consequences for predation risks in heliothermic lizards such as our model species. Altogether, these findings highlight the importance of shelter and wet microhabitat quality in the behavioural responses of ectotherms to future changes in climate conditions (Beck & Jennings, 2003; Moore et al., 2018).

Contrary to our predictions, lizards with access to a wet shelter did not use it more often than the ones with a dry shelter. In addition, despite the high benefits from wet shelter use at night during which significant water loss at rest can occur (i.e. Chabaud et al., 2022), nighttime shelter use increased over time irrespective of treatments. We further predicted a negative relationship between physiological dehydration and frequency of daytime wet shelter use if wet shelter use allows primarily lizards to reduce water losses, whereas a positive relationship was observed in the wet shelter treatment group. That daytime inactivity was higher in more dehydrated animals suggests condition-dependent wet shelter use. These results apparently contradict those of earlier studies of hydroregulation behaviours in reptiles and other ectothermic animals (reviewed in Dezetter et al., 2022; Pintor et al., 2016). For example, Dezetter et al. (2022) found that water-deprived vipers exposed to hot and dry weather conditions used a wet shelter more frequently during daytime and suffered less from drought stress (i.e., less dehydration, body mass loss and muscle wasting) compared to vipers provided with a dry shelter. Nevertheless, unlike our study, vipers in this experiment were placed in climatic chambers with a constant diel temperature cycle such that their body temperature was imposed and trade-offs between thermoregulatory and hydroregulatory activities were

impossible. Instead, our experimental design examines the selection of a wet shelter (i.e., high hydric quality) with a low average temperature relative to the standard thermal preferences of adult common lizards (i.e., low thermal quality). Thus, behavioural selection of a wet microclimate inside the shelter allowed lizards to maintain a better hydration state more efficiently but also involved the selection of a thermally suboptimal microhabitat and had a strong opportunity and energetic cost because it was detrimental to thermoregulatory activities essential for energy intake such as prey capture and assimilation rates (Van Damme et al., 1991). This trade-off between prioritizing basking and foraging activities (above ground activity and accurate thermoregulation versus retreat in a cold site) over hydroregulation (wet shelter use during the day versus above ground activity) may explain why mean shelter use during daytime was not influenced by microclimatic conditions (but see below for a discussion of the effects of food). Furthermore, in our experiment lizards had limited habitat choice options because we were interested in the buffering role of retreat sites rather than habitat selection strategies. If lizards had been provided with both dry and wet shelter, we would predict that lizards would favour the use of wet shelter during inactive phases (Rozen-Rechels et al., 2020), especially when food was present (see below).

Food availability did not influence mean daily thermoregulation effort contrary to predictions of the cost-benefit model of thermoregulation and results of earlier studies, where some reptile species showed signs of thermal depression and decreased basking effort during fasting (Angilletta Jr., 2009; Brown & Griffin, 2005; Gilbert & Miles, 2016; Huey & Slatkin, 1976). The only noticeable effect of food availability on behaviour was a weak positive effect of food intake on daily  $T_b$  values if we replace in our analysis the categorical effect of time with a linear effect of time (linear time effect  $\times$  food treatment:  $F_{1,2521} = 4.54$ ,  $p = 0.03$ ) and a higher decline in daytime  $T_b$  values during the afternoon for food-deprived lizards provided with a dry shelter (see Figure S6 of Supplementary Information). These results indicate that common lizards have limited behavioural acclimation responses to energy imbalance, maybe because fasting was too short or because physiological responses to fasting (enhanced catabolism of lipid stores and muscle wasting in food-deprived lizards) were prioritized over behavioural responses. Finally, none of our findings supported our “food for water hypothesis”. If food consumption represents a significant net gain of water (e.g. Nagy et al., 1991), *ad libitum* food conditions would have potentially reduced plasma osmolality increase during the manipulation or should have reduced the impacts of water restriction on body condition, which was not the case. Similarly, according to our “food for water hypothesis”, behavioural adjustments of

thermoregulation and activity should be greater under food-deprived conditions than under *ad libitum* food conditions, whereas the surprising reverse pattern was observed for daytime shelter use. Instead, the fact that shelter use was more frequent in the presence of a dry shelter and *ad libitum* food could suggest that food consumption and processing require a good hydration status and thus food availability increases reliance on free standing water, as suggested for several snake species and a carnivorous lizard (Lillywhite, 2017; Murphy & DeNardo, 2019; Wright et al., 2013). Therefore, our experiment indicates that food acquisition exacerbates the conflict between thermoregulation and hydoregulation, most likely because behavioural optimization of food capture, processing and digestion processes implies behavioural selection of elevated body temperatures or increased basking effort, which are water costly behaviours. An alternative explanation is that feeding itself induces direct water requirements if prey capture and digestion require a good hydration state (Lillywhite, 2017). Thus, in the presence of food and a dry shelter, lizards should spend more of their activity time inside the shelter to regulate their hydration state than lizards provided with a wet shelter in order to sustain their foraging activities and the food processing and digestion mechanisms. Irrespective of the exact explanations, our results imply that (1) food intake did not play a major, direct role in regulating the hydration status of this species, (2) food intake was not capable of mitigating the body condition effects of water restriction on this species, and (3) that the need to feed and priority of feeding activities implied a greater dependence on microclimatic conditions inside the shelter.

## **Conclusions**

Our factorial experiment was designed to examine thermo-hydoregulation strategies in laboratory conditions where lizards had limited behavioural options including being active or not and retreating inside a shelter or not, which is similar to the kinds of behavioural routines simulated in current mechanistic models of ectotherms (Kearney, 2013). Our results challenge the assumptions of these mechanistic models, which are calibrated on the thermal biology of ectotherms without considering the availability of water resources and the moisture of microhabitats (Sinervo et al., 2010). They also suggest that water balance regulation may interact with energy regulation and foraging behaviours in unexpected ways. In particular, our data indicate that a potential water gain from feeding could be offset by direct water requirements induced by the physiological mechanisms of digestion or by indirect water requirements associated with foraging or assimilation processes. Our findings demonstrate the urgent need to take into account behavioural interactions between thermoregulation and



hydreregulation as well as the dual effect of warming and drought events in future mechanistic modelling. In this context, future models will have to embrace the natural complexity of microclimatic conditions and foraging strategies of ectotherms. In particular, a better understanding of the buffering role of microclimatic conditions inside natural shelters used during phases of inactivity will improve our understanding of the ecological responses of terrestrial ectotherms to climate change (Moore et al., 2018).

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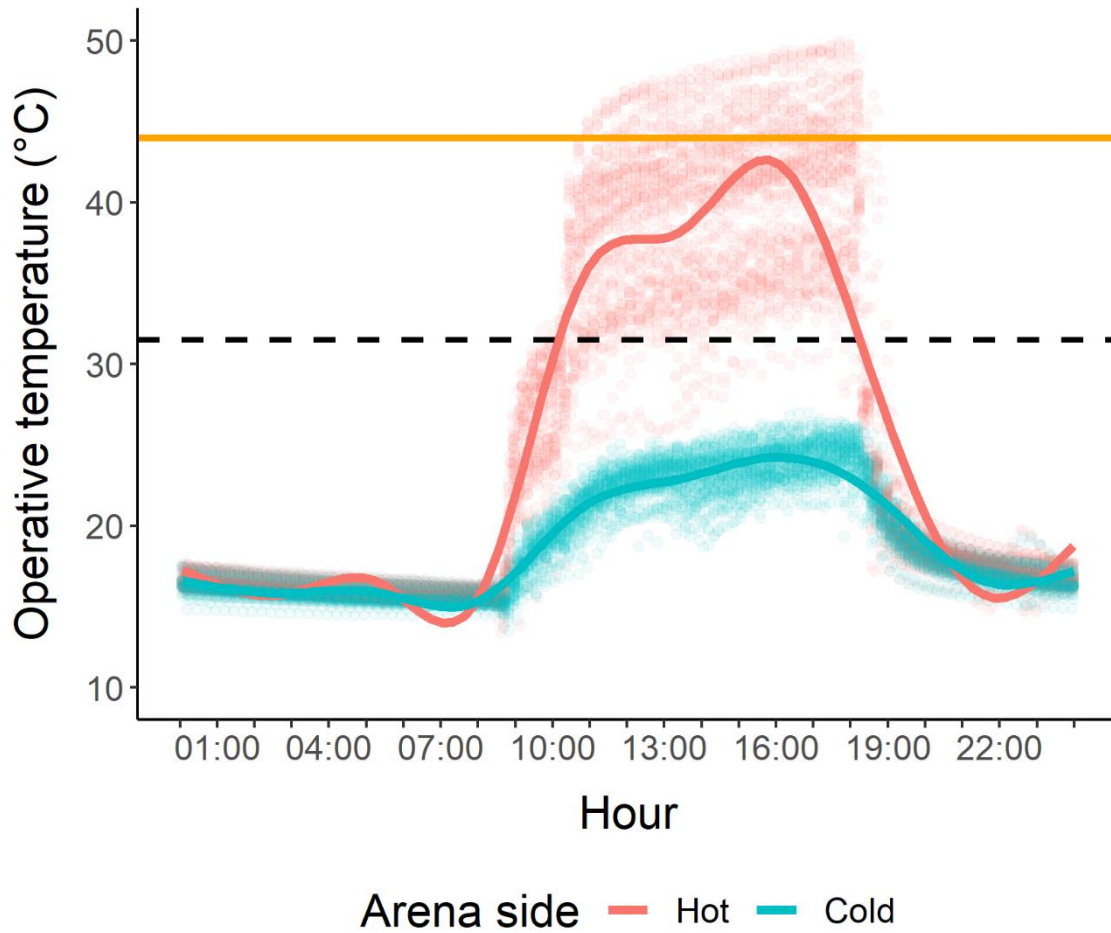
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## Supplementary Information 1

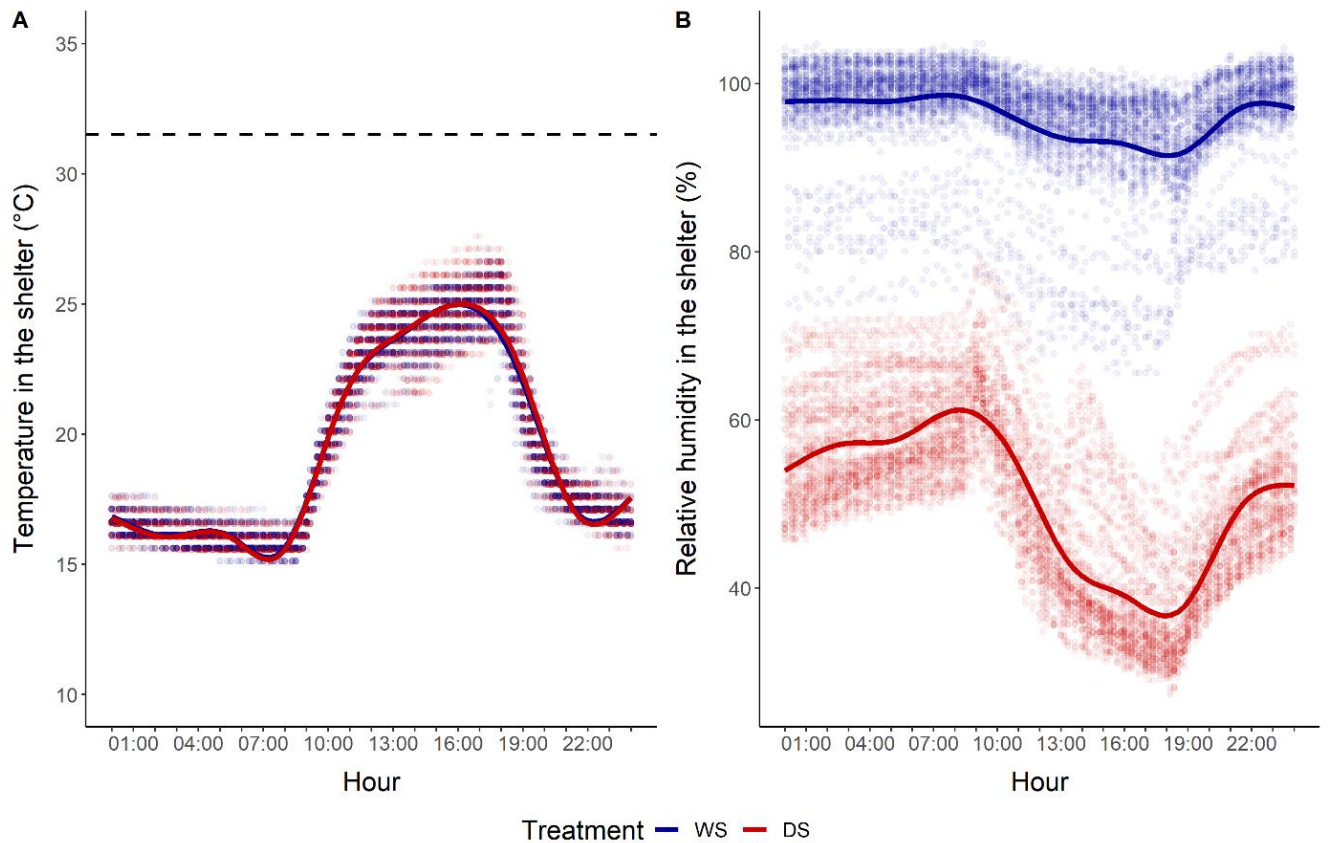
Figure S1

Daily variation of operative temperature on the hot and cold side of the thermal gradient. Jittered points correspond to raw operative temperature values. Smoothing with a generalized additive model was applied to visualize the nonlinear patterns (solid blue and red line). Thermal preferences (black dashed line) and upper critical thermal limit (orange solid line) of *Zootoca vivipara* (Gvoždík & Castilla, 2001) are represented.



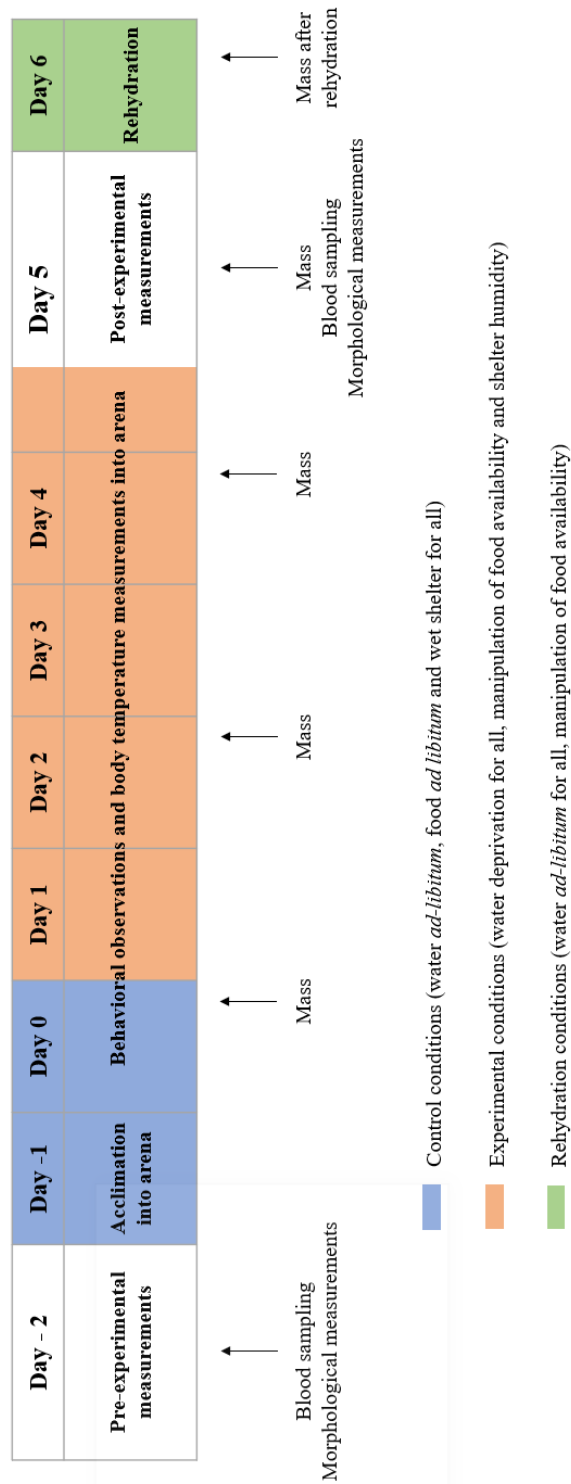
**Figure S2**

Daily variation of temperature (A) and relative humidity (B) in shelters as a function of shelter treatment (WS: wet shelter, DS: dry shelter). Jittered points correspond to raw values, red and blue lines are predictions from a generalized additive model. Thermal preference of *Zootoca vivipara* (Gvoždík & Castilla, 2001) is represented by a black dashed line. Mean temperature in the shelter did not differ significantly between dry and wet treatments ( $F_{1,16123} = 2.28, p = 0.95$ ).



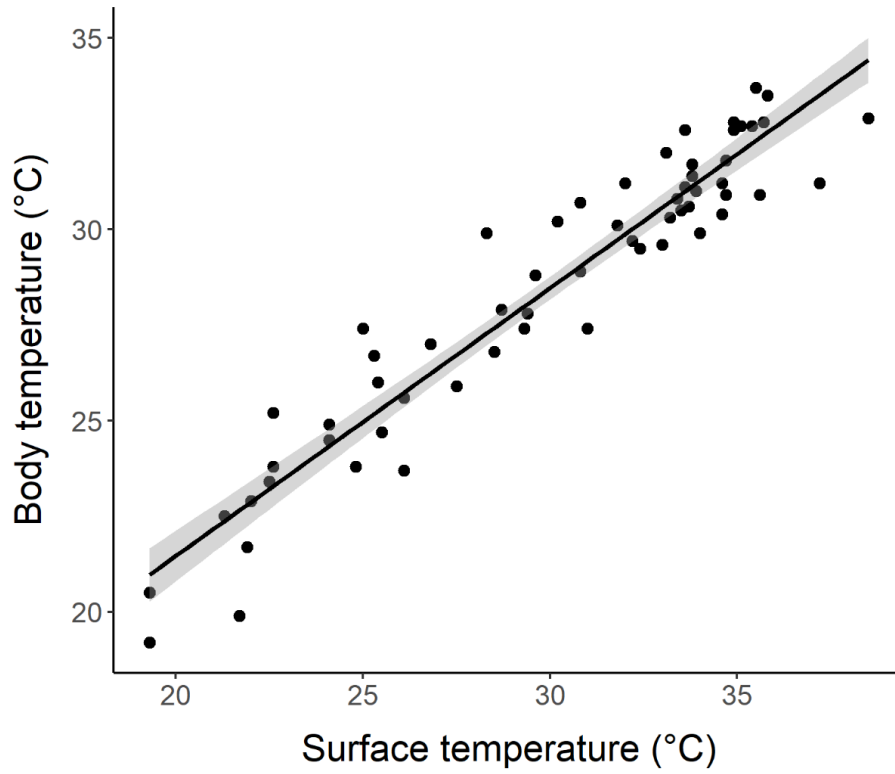
**Figure S3**

Chronology of the experiment for each experimental batch.



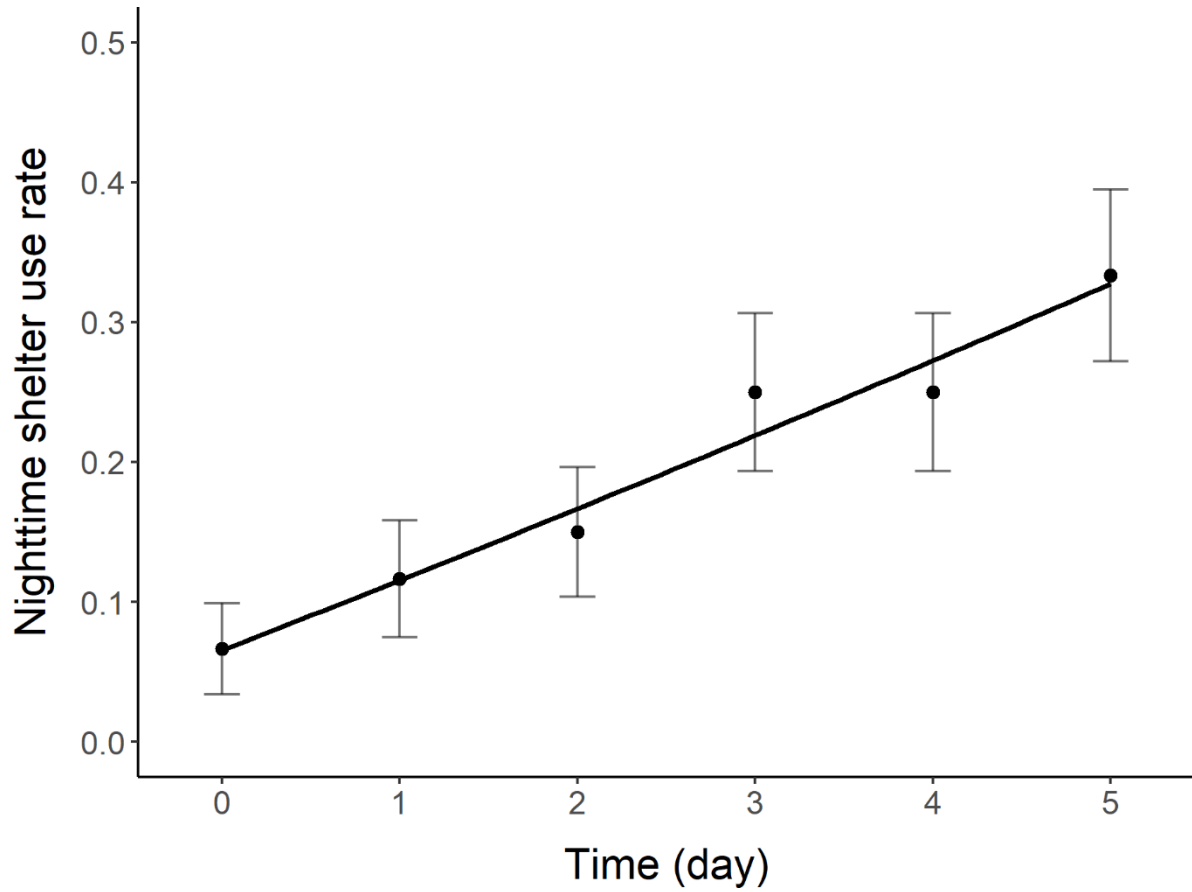
### Figure S4

Relationship between internal body temperature measured with a K-type thermocouple inserted in the cloaca and surface body temperature measured with an infrared thermometer. The linear regression (with 95% confidence interval) is plotted against raw data.



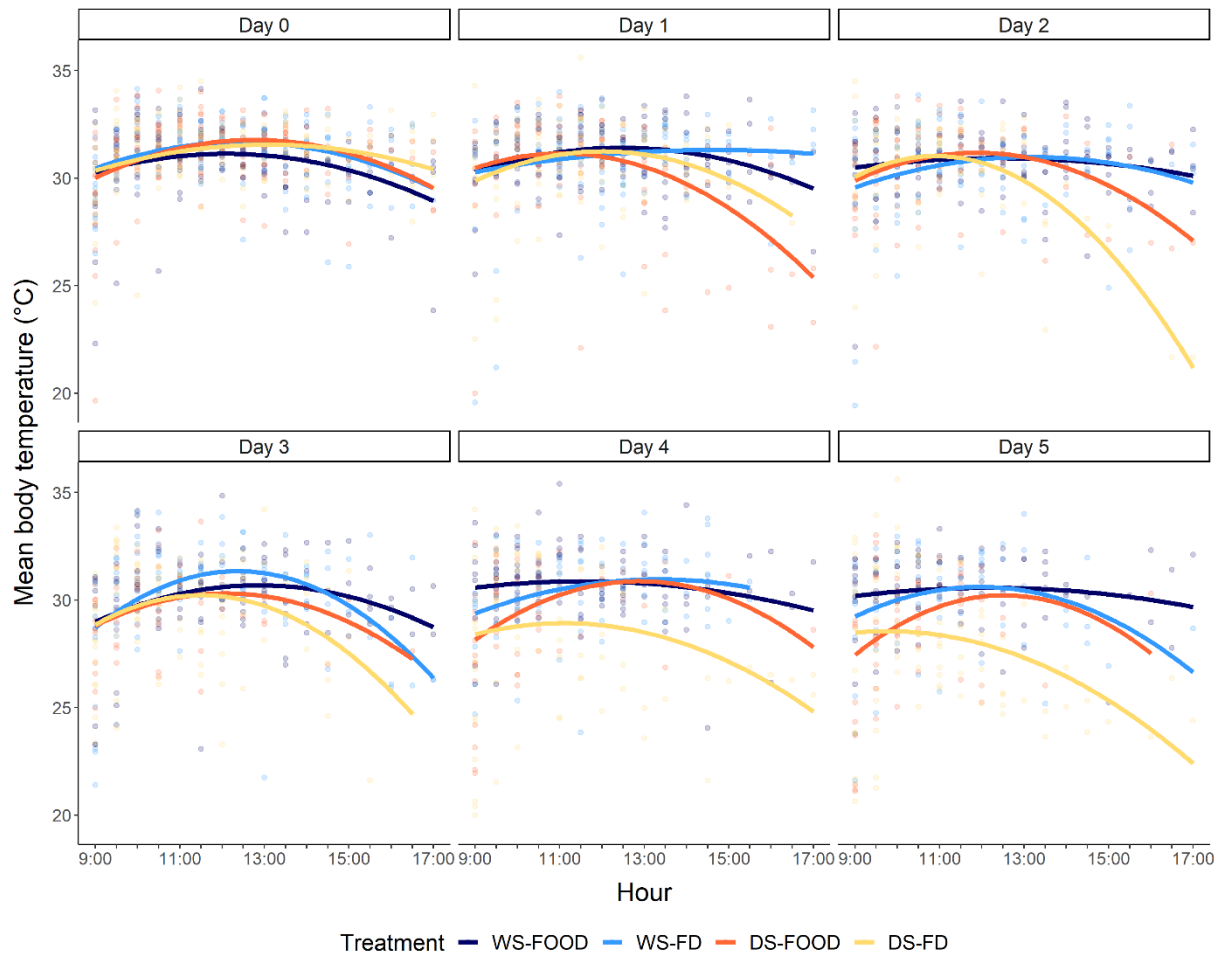
**Figure S5**

Nighttime shelter use as a function of time (number of days). Points and error bars represent average nighttime shelter use ( $\pm$  SE) and the solid line represent the average nighttime shelter use adjusted with a linear time effect. Water restriction begins on day 1.



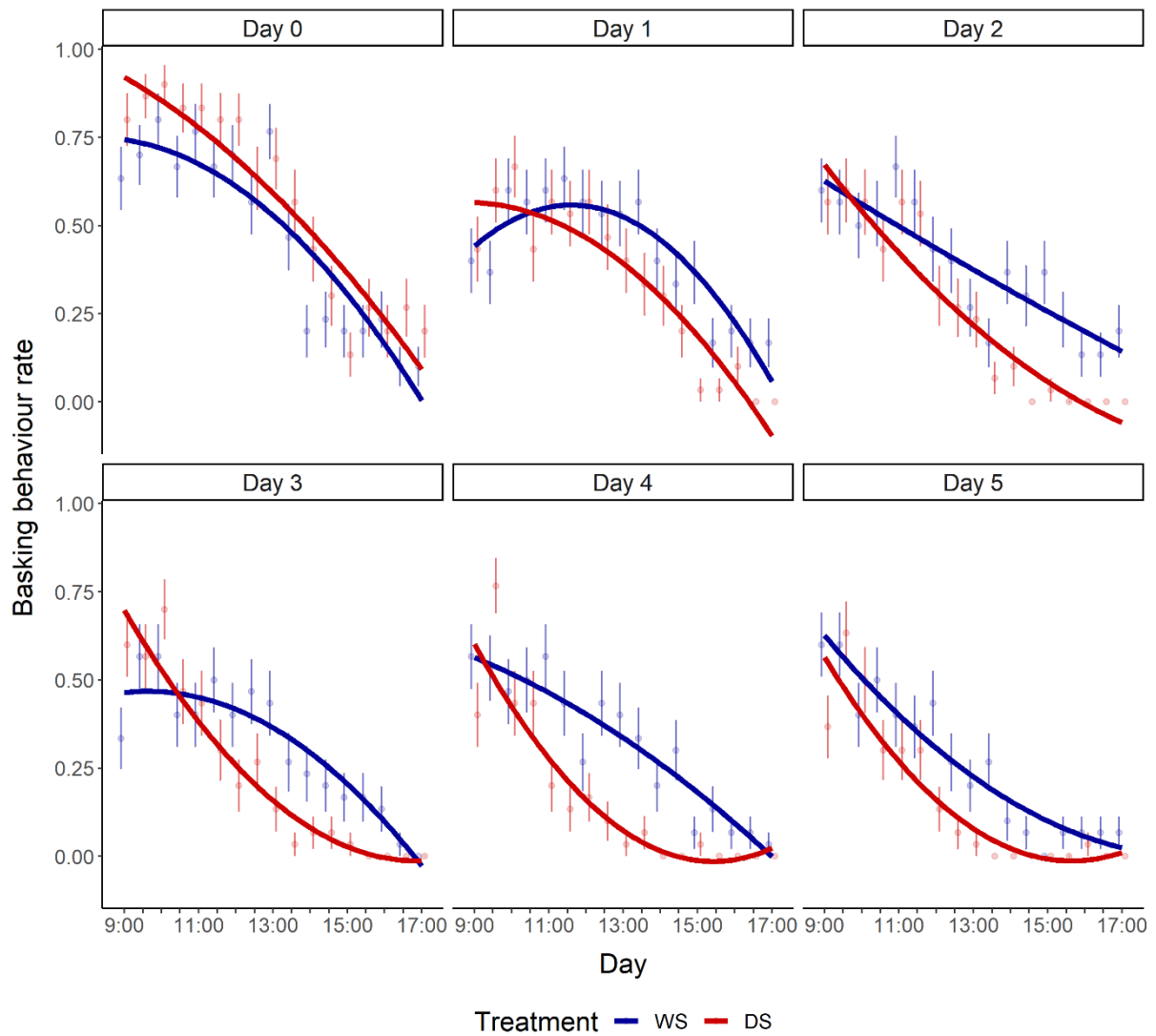
**Figure S6**

Interactive effects of shelter and food treatments on body temperatures as a function of time (number of days since start of the experiment) and daytime (hour of the day). WS-FOOD (dark blue): wet shelter and *ad libitum* feeding, WS-FD (light blue): wet shelter and food deprivation, DS-FOOD (orange): dry shelter and *ad libitum* feeding, DS-FD (yellow): dry shelter and food deprivation. Points represent  $T_b$  and the solid lines represent the average  $T_b$  adjusted with a quadratic daytime effect. Water restriction begins on day 1.



**Figure S7**

Effects of shelter treatment on basking behaviour as a function of time (number of days since start of the experiment) and daytime (hour of the day). Colours represent lizards with access to wet shelter (WS (dark blue)) or dry shelter (DS (red)). Points and error bars represent average basking behaviour rate ( $\pm$  SE) and the solid lines represent average basking behaviour rate adjusted with a quadratic hour effect. Water restriction begins on day 1.







## **Chapitre 4) Prey consumption does not restore hydration state but can mitigate the energetic costs of water deprivation in a mesopredator lizard**

*Submitted for publication in Journal of Experimental Biology*

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## **Abstract**

To cope with limited drinking water availability in their environment, terrestrial animals have developed numerous behavioral and physiological strategies including maintaining an optimal hydration state through dietary water intake. Recent studies performed in snakes, which are carnivorous reptiles with a specialized diet, suggest that benefits of dietary water intake are negated by hydric costs of digestion. Most lizards are generalist insectivores that can shift their prey types, but firm experimental demonstration of dietary water intake is currently missing in these organisms. Here, we performed an experimental study in the common lizard, a keystone mesopredator from temperate climates exhibiting a great diversity of prey in its mesic habitats, in order to investigate the effects of food consumption and prey type on physiological responses to water deprivation. Our results indicate that common lizards cannot improve their hydration state through prey consumption, irrespective of prey type, suggesting that they are primarily dependent upon drinking water. Yet, high-quality prey consumption reduced the energetic costs of water deprivation, potentially helping lizards to conserve a better body condition during periods of limited water availability. These findings have important implications for understanding the physiological responses of ectotherms to water stress, and highlight the complex interactions between hydration status, energy metabolism, and feeding behavior in insectivorous lizards.

Keywords: physiology - lizards – water balance – dietary water – metabolites - reptiles

## 1. Introduction

As climatic conditions shift towards longer and more frequent warm and dry spells in many regions (IPCC 2021), it is critical to understand and quantify the contributions of different water sources in wild animals. Optimal hydration state can be maintained by drinking free-standing water (Karasov & del Rio, 2007), food consumption (dietary water intake, Nagy & Knight, 1994), or internal metabolic production (metabolic water, Bailey, 1923; Jindra & Sehnal, 1990). Free-standing water is the most widespread, primary source of water intake in animals, but it is not continuously available in many terrestrial ecosystems and most organisms have developed strategies to cope with limited water availability. For example, water requirements can be met through dietary shifts (Leinbach, McCluney, & Sabo, 2019), utilizing internal water reservoirs (e.g., urinary bladder, Davis & DeNardo, 2007), or even harvesting water from fog (Seely, 1979). Conversely, water loss can be minimized through behavioral changes in activity patterns (Bissonette, 1978) or microhabitat use (Dupoué *et al.*, 2015; Dezetter, Le Galliard, & Lourdais, 2022), but also through osmoregulatory strategies (e.g., nitrogen excretion via increased uric acid production, Braun, 1999). Finally, some species can tolerate hyperosmolality and deviate from homeostatic set-points to cope with water constraints (Peterson, 1996; Bradshaw, 1997). These strategies all have implications for the capacity of species to survive in more arid environments (Cloudsley-Thompson, 1975), but have been little investigated so far in non-arid adapted species.

The strong dependence of water budgets on dietary water intake has been shown in some animals living in semi-arid or arid environments characterized by low annual rainfall, high temperatures, and recurrent drought episodes (Nagy, 2004). For example, many herbivorous desert vertebrates feed on plant tissues or seeds to sustain their standard activity and water balance even when water is not available in the environment (in mammals, Nagy & Gruchacz, 1994; in birds, Tieleman, 2002; in turtles, Nagy & Medica', 1986; in lizards, Minnich & Shoemaker, 1970). Such dietary water intake is thought to provide a considerable advantage in these extreme environments and some species display adaptive shifts in food preferences toward resources with higher water content during the dry season (Karasov, 1983). However, recent studies in carnivorous squamate reptiles (i.e., lizards, snakes, and amphisbaenians), including some living in arid environments, suggest that they do not rely on dietary water intake due to the lower water content of their prey and the important protein content that increases the hydric cost of digestion (Wright, Jackson, & DeNardo, 2013; Lillywhite, 2017). Even in arid environments, these species thus do not receive net hydric benefits from their diet (Murphy &

DeNardo, 2019). Indeed, fluid secretion is required during digestion to aid in transport and absorption of nutrients, and this hydric cost can be particularly important when carnivorous reptiles are already dehydrated (Murphy & DeNardo, 2019). Feeding may also involve behavioral changes that increase water loss rates (such as locomotor activity during foraging and basking during digestion) and trade-offs can occur between food acquisition and water loss (Gregory, Crampton, & Skebo, 1999). In addition, carnivorous reptiles feed intermittently and it has been suggested that this may increase the hydric cost of digestion and reduce opportunities for dietary shifts towards prey with higher water contents (Lillywhite, 2017).

Currently, there is a limited knowledge about dietary water intake and hydric costs of digestion in insectivorous reptiles, which include the vast majority of lizards (see Table 1; Meiri, 2018), whereas several species are also omnivorous, eating plant material occasionally (Pianka, 1973; Cooper Jr & Vitt, 2002). In addition, it remains unknown if inferences drawn from studies of arid adapted species apply to species from moister tropical or temperate zones, where free-standing water is more regularly available. Most insectivorous lizards are generalist mesopredators with a non-intermittent diet dominated by invertebrates such as insects, spiders, or other arthropods (Meiri, 2018). The invertebrate prey of these lizards are highly variable in size, digestibility, and macro- and micro-nutrient content including water (McCluney & Date, 2008). Generalist lizards can thus shift their diet to meet different demands depending on prey availability and resource expenditures across the activity season or between different life stages (Bestion *et al.*, 2019). Previous work has focused on the energetic quality of food and its adequation to optimal diet models based on energetic constraints (Stamps, Tanaka, & Krishnan, 1981; Dearing & Schall, 1992), but very few studies have investigated dietary shift due to water constraints (but see Mautz & Nagy, 1987). Yet, when preys have different water and protein content or different digestibility, this may influence the dietary water intake from different preys, leading to dietary shifts caused by water balance instead of energy balance. For example, if free-standing water is limited in the environment, insectivorous lizards might shift their diet towards high-water to maintain water balance, which can impact the population dynamics and trophic ecology of these species (Bestion *et al.*, 2015). Thus, an understanding of the net dietary water intake and hydric costs of digestion of insectivorous lizards should include the possibility of different prey types that would be representative of their standard diet. To our knowledge, no study has examined if different prey types are more or less effective at maintaining water balance in these organisms.

Water deprivation induce several physiological responses to restore hydration state (lipolysis, reduced hepatic acid synthesis, or protein catabolism to release bound water) as previously demonstrated in birds, lizards, and snakes (Gerson & Guglielmo, 2011; Bruschi *et al.*, 2018). These responses are known to induce energy and structural costs with altered body reserves and muscle condition (Dezetter *et al.*, 2021; Bruschi *et al.*, 2022). Therefore, besides the direct effect of diet on the water budget, we need to consider the whole-organism metabolic responses according to different prey types, since food might be important to recoup these energetic and structural costs of water deprivation. Here, we designed a laboratory experiment to test if consumption of different prey types can compensate for water deprivation. We investigated these responses in the common lizard (*Zootoca vivipara*, Lichtenstein 1823), a generalist mesopredator with a widespread distribution across Eurasia, which is dependent on cool and moist environments inside humid grasslands and bogs. In these natural habitats, the common lizard is a keystone mesopredator due to its great diversity of invertebrate prey and its abundance where it can reach densities as high as 1,000 lizards per ha (Avery, 1971; Bestion *et al.*, 2015). Earlier field and laboratory studies have suggested that the species is dependent on free-standing water to maintain water balance (Grenot *et al.*, 1987; Lorenzon *et al.*, 1999; Rozen-Rechels *et al.*, 2020). Water deprivation causes a range of systemic responses associated with a reduction of body mass, proximal tail width (where lipids are stored, Chapple & Swain, 2002), and limb thickness (due to muscle wasting, Bruschi *et al.*, 2018). However, no previous study has investigated the link between diet, water balance, and body condition.

Herein, we tested for the occurrence of dietary water intake by comparing changes in plasma osmolality of mildly dehydrated lizards following the consumption of three distinct types of invertebrate prey with contrasted nutrient and water contents (crickets, spiders, and woodlice). These preys represent the trophic diversity of prey in natural communities, (see Bestion *et al.*, 2015). While crickets and spider have high water content, the woodlice should provide less water and energy. These lizards were fed but had no access to water during the 6 days of the experiment, in a temperature-controlled room. They were compared to two groups of unfed lizards: one “hydrated control” group with no food but *ad libitum* access to free-standing water and one “dehydrated control” group without food or water. We assessed plasma osmolality before and after meal consumption to get a direct indicator of the response: a decrease in plasma osmolality indicates that lizards can compensate for a lack of drinking water by consuming food, whereas an increase in plasma osmolality indicates that meal consumption (including secretion, digestion, absorption, and motility) has a non-negligible hydric cost

(Wright, Jackson & DeNardo, 2013). We also tracked plasma circulating metabolites associated with catabolism and fasting in reptiles, including glucose liberated from stored glycogen, triglycerides stored in adipose tissues and exported from the liver during fasting, and ketone bodies produced by the breakdown of fatty acids in the liver during periods of food restriction (McCue, Lillywhite, & Beaupre, 2012; Price *et al.*, 2013). In particular, the brain, unlike other organs, can only use glucose and ketone bodies to function, which makes them particularly essential during food restriction (Price, 2017). We also monitored morphology over time (tail and leg width) as indicator of structural mobilization. Using this experimental design, we tested the following hypotheses:

- 1) If lizards can extract dietary water, we predict that experimental groups with access to food would be more similar to the hydrated control than to the dehydrated control.
- 2) On the contrary, if eating represents a net hydric cost, fed lizards will have elevated plasma osmolality compared to the dehydrated control.
- 3) Prey characteristics should have an impact on the response. Notably digestively “costly” preys (hard exoskeleton) such as woodlice should provide limited energy and water benefits. Within the same prey type, prey hydration should also influence the response.

## **2. Material and methods**

### 2.1. Study species

The common lizard *Zootoca vivipara* is a small lacertid lizard (Reptilia: Lacertidae) with a wide Eurosiberian distribution (Surget-Groba *et al.*, 2006). It inhabits cold and wet habitats, such as humid grasslands and bogs, and is highly sensitive to water deprivation due to high standard water loss rates (Lorenzon *et al.*, 1999; Massot *et al.*, 2002; Dupoué *et al.*, 2017). The common lizard is a mesopredator in its food web with a diet of various arthropods (hexapods, spiders, isopods, annelids) ranging in size from 1 to 9 mm (Avery, 1996), which includes decomposers, herbivorous, and predatory species (Bestion *et al.*, 2019). Common lizards used in this study were captured in semi-natural populations maintained in 25 outdoor enclosures in CEREEP-Ecotron IleDeFrance in Saint Pierre lès Nemours, France (48°17'N, 2°41'E). Experiments were carried out in accordance with institutional guidelines, under license from the French Ministry of the Environment (permit APAFIS#25281-2020042915424095).

## 2.2. Housing and acclimation

We captured 128 lizards by hand in June 2020 and brought them back to a laboratory located nearby. We selected only sexually mature males based on external morphology, body size, and known age. Upon capture, one person measured snout-vent length (SVL, mean=59.3 ± 6.5 mm SD) and total length (TL) to the nearest millimeter with a plastic ruler as well as body mass (BM) to the nearest milligram with an electronic balance (mean=4.19 ± 1.62 g SD). Animals were then kept separated in individual terraria (18×11×12 cm) with sterilized peat substrate, a basking rock, a shelter buried in the substrate and a water cup with *ad libitum* access to water. During captivity, we provided all lizards with the same thermal conditions in a temperature-controlled room (23°C for 9h per day 09:00-18:00, and 16°C at night) in addition to heating lamp and UV above their terrarium during daytime time (33°C below heating lamp). Lizards were fed with live crickets (*Acheta domesticus*) every two days and had water sprayed on the sides of their cages three times a day during acclimation.

## 2.3. Measurements and blood sampling:

After one week of acclimation, we collected morphology data of hydrated and fed lizards (referred to as T0 measurements). During the same day, they were weighed with an electronic balance and photographed with a digital scanner (ventral view, CanoScan LiDE 700F, Canon) to measure tail width, a trait linked to fat and muscle storage of the tail. Tail width was measured at the 7<sup>th</sup> and 10<sup>th</sup> subcaudal scales by processing the digital images with the software ImageJ (Schneider, Rasband & Eliceiri, 2012). We also assessed the transverse muscle thickness of the right hind leg at the mid region of the upper limb with digital calipers (ID-C1012BS, Mitutoyo, Japan, ±0.01 mm). These three morphological variables (body mass, tail width, and hindlimb width) were used as indicators of dehydration and well-being during the whole study. We also took a blood sample from the post-orbital sinus using 1 to 2 micro-capillary tubes (ca. 20 µl whole blood). In the laboratory, samples were centrifuged at 11,000 rpm for 5 min to separate plasma from red blood cells. Plasma samples (approx. 10 µl) were immediately frozen at -28°C until osmolality assays (see section 2.5).

## 2.4. Experimental design

In order to investigate the effects of food consumption on water and energy balance in mildly dehydrated lizards, we first exposed all lizards to a moderate water restriction for 10 days (Fig 1A). To do so, we removed the water cup from terraria and reduced water spray to once every two days while feeding lizards every two days. Water spray only provided lizards a few milliliters of water that dried in less than 30 minutes (no water at all during 6 days is fatal for

adults in this species, pers. obs.). At the end of the 10<sup>th</sup> day, we weighed lizards and measured tail and muscle width as indicated above (referred to as T1 measurements). We collected blood samples using 2 to 3 micro-capillary tubes (ca. 30-40  $\mu$ l whole blood) and stored plasma samples like indicated above until osmolality and metabolite assays (see section 2.5).

We selected three types of prey known to be part of this species diet and with distinct trophic roles. First, we provided some lizards with crickets *Acheta domesticus*, an herbivorous insect that can be purchased at reptile breeding shops. Crickets were kept with dry food and with or without water in a climatic chamber at 25°C based on earlier studies showing that such conditions can trigger dehydration (McCluney & Date, 2008). We also wanted to compare a same type of prey but with different water content, in order to test the quality of prey in addition to prey type. To do so, we provided lizards either with “hydrated” crickets (held with access to water) or with “dehydrated” crickets (held for at least a week without access to water). Second, we provided other lizards with woodlice (several species including *Armadillum vulgare*, *Porcellio scaber*, *Philloscia muscorum* and *Oniscus asellus*), detritivorous isopods which were collected in the same outdoor enclosures as the lizards. Woodlice were kept in a dark, humid box with leaf and compost until being provided to the lizards. Third, we provided some lizards with spiders (several species of Lycosidae or wolf spiders), carnivorous arachnids collected by hand in the nearby forest plot as well as in outdoor enclosures. Spiders were collected the day before feeding the lizard and kept alone with humid cotton and no food prior to being used. The three species of prey are different in their macronutrient components (proteins, carbohydrates and lipids, see Reeves *et al.*, 2021) and differences in those ratios can inform us about the hydric cost of digestion depending on prey type.

To measure prey water content, invertebrates were first weighed alive individually with a high precision scale ( $\pm 0.01$  mg). Then, they were put in the freezer at -20°C for a few hours to be killed, before being dried in a lab oven at 60°C for 24 hours, and weighed again to determine dry mass. Water content was calculated as the difference between wet mass and dry mass. In addition, energy content was determined with dry aliquots (0.5-1.1g) by using an isoperibol bomb calorimeter (Parr 6200), with benzoic acid as a standard. Woodlice differed significantly in water content and energetic content, being of poorer quality, while the other prey types were similar (Table 2).



We separated lizards into six different treatments for the next six days (Fig 1A) including :

- Group 1 : “hydrated control” where lizards had access to *ad libitum* water but no food,
- Groups 2 to 5 : groups of lizards that did not have access to *ad libitum* water but were provided 200 mg per day of either hydrated crickets (2), dehydrated crickets (3), woodlice (4), or spiders (5)
- Group 6: “dehydrated control” where lizards did not have access to *ad libitum* water or food.

We provided the same quantity of each prey type (200mg) which corresponded to similar energy intake for crickets and spiders but not for woodlice (Table 2). We measured the body mass of lizards every two days, and we weighed any remaining food in the terrarium to assess how much was eaten by each individual (Table 2). On the 7<sup>th</sup> day, we measured body mass, leg and muscle width (T2 measurement), and collected a blood sample to measure plasma osmolality and metabolites. All lizards were then removed from the experiment, fed and provided water *ad libitum* for 2-3 days, and released into the outdoor enclosures.

**Table 2:** Water content and nutritional quality (mean  $\pm$  SD) of invertebrates used in the experiment. N=20 individuals for Water content, N = number of pools used for energy content, each pool containing tens of individuals, and N = number of lizards in each food treatment for quantity of food eaten. Quantity of food eaten by each treatment groups during the 6 days of manipulation 1,2 g being provided in total. \*\*\* indicates that the group is significantly different from the others.

|                               | <b>Hydrated Crickets</b>    | <b>Dehydrated Crickets</b>  | <b>Spiders</b>              | <b>Woodlice</b>                    |
|-------------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------------|
| <b>Water content</b>          | 75.25 $\pm$ 3.99 %<br>N=20  | 72.24 $\pm$ 2.27 %<br>N=20  | 73.27 $\pm$ 4.83 %<br>N=20  | 65.58 $\pm$ 1.74 %<br>N=20 ***     |
| <b>Energy content</b>         | 6.23 $\pm$ 0.29 kJ/g<br>N=4 | 6.52 kJ/g<br>N=1            | 6.34 $\pm$ 0.51 kJ/g<br>N=2 | 3.92 $\pm$ 0.11 kJ/g<br>N=3 ***    |
| <b>Quantity of food eaten</b> | 0.98 $\pm$ 0.23 g<br>N = 21 | 1.02 $\pm$ 0.14 g<br>N = 22 | 1.01 $\pm$ 0.15 g<br>N = 21 | 0.78 $\pm$ 0.08 g<br>***<br>N = 21 |

## 2.5. Plasma assays

First, plasma osmolality was determined using a vapor pressure osmometer (Vapro 5600, ELITechGroup) with the protocol described in Wright *et al.* (2013) adjusted to small plasma volumes (Dupoué *et al.*, 2017). Before analyses, plasma was diluted (1:3) in standard saline solution (280 mOsm kg<sup>-1</sup>) to obtain duplicates for each sample (CV<0.4%). Normosmic plasma osmolality values (280-320 mOsm kg<sup>-1</sup>) indicate that lizards were hydrated, whereas hyperosmotic values (> 350 mOsm kg<sup>-1</sup>) indicate that lizards were moderately to sub-clinically dehydrated (Dupoué *et al.*, 2018). Second, we measured plasma concentrations of ketone bodies  $\beta$ -Hydroxybutyrate ( $\beta$ -HB) with a colorimetric method ( $\beta$ -Hydroxybutyrate Ketone Body Colorimetric Assay Kit, ref n°700190, Cayman Chemical, USA).  $\beta$ -HB are carbohydrates that are produced in the liver as a byproduct of the catabolism of fatty acid  $\beta$ -oxidation during fasting periods in a process known as ketosis.  $\beta$ -HB can be used as an energy source for the brain during periods of starvation and thus provide information on the nutritional status (McCue, 2008; McCue *et al.*, 2012). Here, plasma samples were diluted to 1:8 (using 8  $\mu$ l of plasma) in the dilution buffer of the assay kit. Absorbance was measured at 450 nm and then transformed to concentration values using a dilution range provided by the kit. We performed duplicates on a few samples to measure a coefficient of variation within plates (CV<sub>within</sub>=7%) and between plates (CV<sub>between</sub>=11%) Third, we measured levels of circulating triglycerides in plasma by a colorimetric assay (Triglyceride Colorimetric Assay kit, ref n°10010303, Cayman Chemical, USA) using 2.5  $\mu$ l of plasma diluted at 1:4 in provided dilution buffer. This method quantifies the total triglycerides using a chain of three enzymatic reactions ending up in hydrogen peroxide production, which is converted into quinoneimine dye quantified by absorbance at 540 nm (CV<sub>within</sub>=12% ; CV<sub>between</sub>=13%). Finally, we measured glucose concentration in the plasma with a colorimetric assay (Glucose Colorimetric Assay Kit, ref n°10009582, Cayman Chemical, USA). We used 1.5  $\mu$ l of plasma diluted at 1:13 in the provided dilution buffer as the glucose concentrations were very high compared to kit sensibility. After three chemical reactions, the product of glucose is pink and quantified by absorbance at 500 nm (CV<sub>within</sub>=4% ; CV<sub>between</sub> > 30% so we only compared samples measured on the same kit).

## 2.6. Statistical analyses

All statistical analyses were performed using R statistical software (version 3.6.3; R Core Team 2020). First, we checked plasma osmolality values during the first phase of our manipulation to verify that lizards were mildly dehydrated and dehydration was similar among treatment groups. This test was performed with an analysis of variance of the intra-individual change in plasma osmolality from T0 to T1. There was no difference among groups ( $F_{5,120}=0.89$ ,  $p=0.49$ ) since all lizards had similar increases in plasma osmolality (mean  $\pm$  SE:  $41.08 \pm 3.86$  mOsm  $\text{kg}^{-1}$ ). Then, to assess the effects of treatments on morphology, plasma osmolality, and metabolites over the course of the experiment, we performed independent linear models for each response variable. We calculated intra-individual changes as the difference between T1 and T2 (referred to as  $\Delta$ variable) and included the fixed effects of treatment groups and initial value of the response variable in a linear regression model fitted with the *lm* procedure. Prior to statistical tests, we used diagnostic plots of residuals to verify the model assumptions with regard to the normality and homogeneous variance of residuals (Heumann, Schomaker, & Shalabh, 2016). *Post-hoc* tests of differences among groups were performed using the *multcomp* package (Hothorn *et al.*, 2016). All results are reported as means  $\pm$  SE unless otherwise stated. As we noticed a difference in the amount of food consumed among treatment groups with access to food ( $F_{3,83}=10.22$ ,  $p<0.0001$ ), we conducted a second set of analyses with fed lizards using the total amount of food consumed during the manipulation period as a covariate.

## **3. Results**

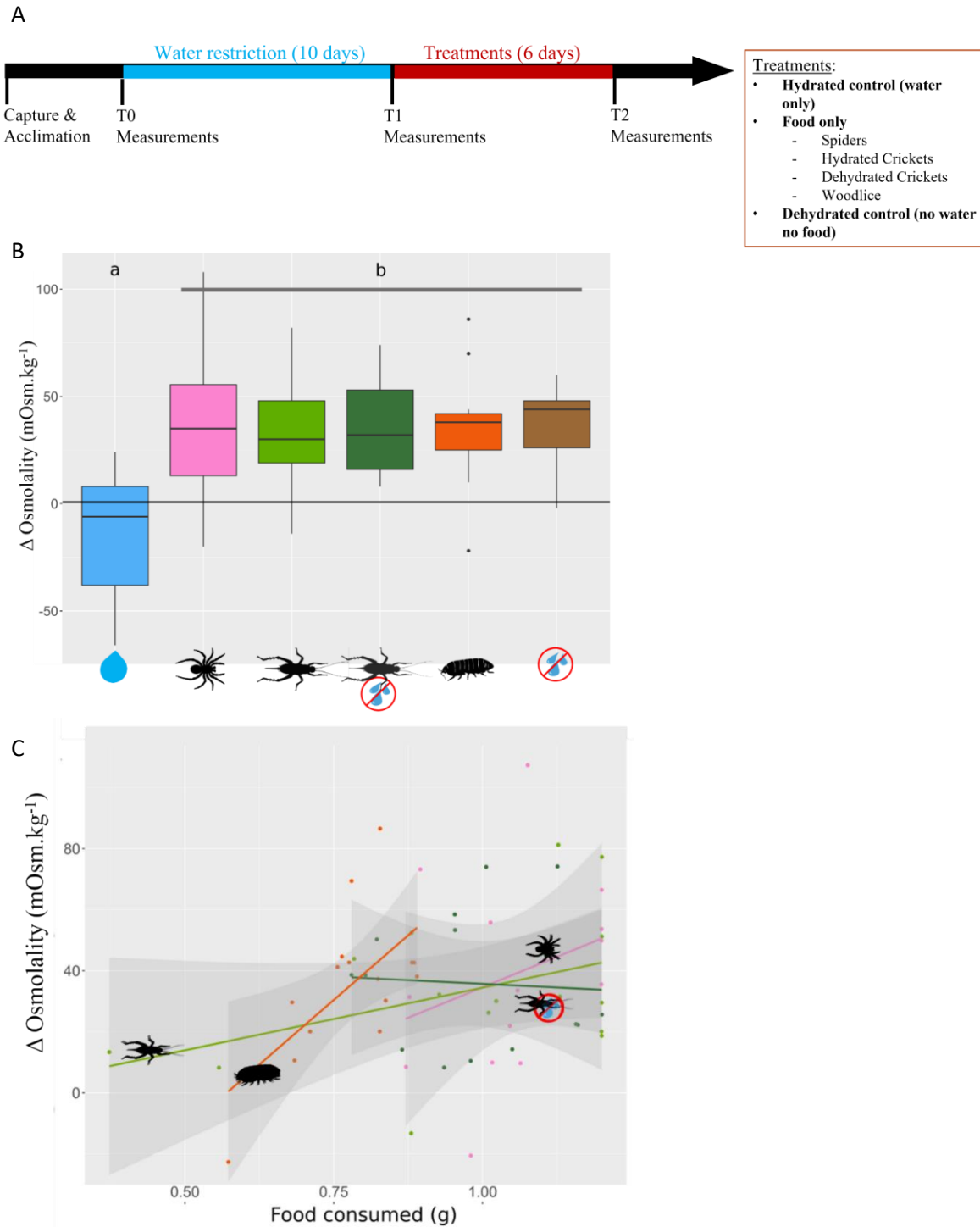
### 3.1. Dehydration and water balance

Treatment groups had a significant effect on  $\Delta$ osmolality ( $F_{5,120}=11.27$ ,  $p<0.0001$ ; Fig 1B). Lizards in the hydrated control group restored their water balance to some extent, whereas plasma osmolality increased in all other groups including lizards provided with food, irrespective of their diet. Additionally, among fed lizards, plasma osmolality increased more when lizards consumed more food across all treatment groups, as shown by the mild positive correlation between food consumption and plasma dehydration during the manipulation ( $F_{1,79}=3.92$ ,  $p=0.05$ ; Fig 1C).

### 3.2. Morphological changes

Mean initial body mass was  $3.81 \pm 0.05$  g with no difference among treatment groups ( $F_{5,123}=0.64$  ;  $p=0.67$ ), but body mass change between T1 and T2 ( $\Delta$ BM) differed strongly among treatment groups ( $F_{5,128}=34.23$ ;  $p<0.0001$ ; Fig 2A). Lizards in hydrated control group

lost less body mass than other treatment groups (Tukey *post-hoc* tests, all  $p < 0.005$ ). Lizards provided with spiders or crickets lost less mass than those provided with woodlice or dehydrated control ( $p < 0.0001$ ). Of the fed lizards, those provided with woodlice lost the most body mass and it was not statistically different than in lizards deprived of free-standing water and food ( $p = 0.29$ ; Fig 2A). With regard to tail width, it did not change on average in hydrated control lizards or those provided with spiders or crickets (contrast:  $p > 0.3$ ; Fig 2B), whereas a significant and similar reduction in tail width was observed in the dehydrated control and lizards fed with woodlice (respectively, contrasts:  $\beta = -0.24$ ;  $p = 0.01$  and  $\beta = -0.26$ ;  $p = 0.02$ ; Fig 2B). At the same time, hindlimb width decreased on average in all lizards (mean  $\Delta$ hindlimb =  $-0.15 \pm 0.009$  mm, Fig 2C). Hindlimb loss width was similar and low in hydrated control lizards and those provided with spiders or hydrated cricket (Tukey *post-hoc* tests : all  $p > 0.2$ , Fig 2C). Meanwhile,  $\Delta$ hindlimb width was strongest in lizards from dehydrated control and in lizards provided with woodlice and dehydrated crickets (Tukey *post-hoc* tests :  $p < 0.05$ ). The amount of food consumed by each lizard did not correlate with the morphological changes ( $\Delta$ BM  $F_{1,82} = 0.66$ ;  $p = 0.42$ ;  $\Delta$ tail  $F_{1,76} = 1.65$ ;  $p = 0.21$ ;  $\Delta$ hindlimb  $F_{1,80} = 0.0004$ ;  $p = 0.98$ ).

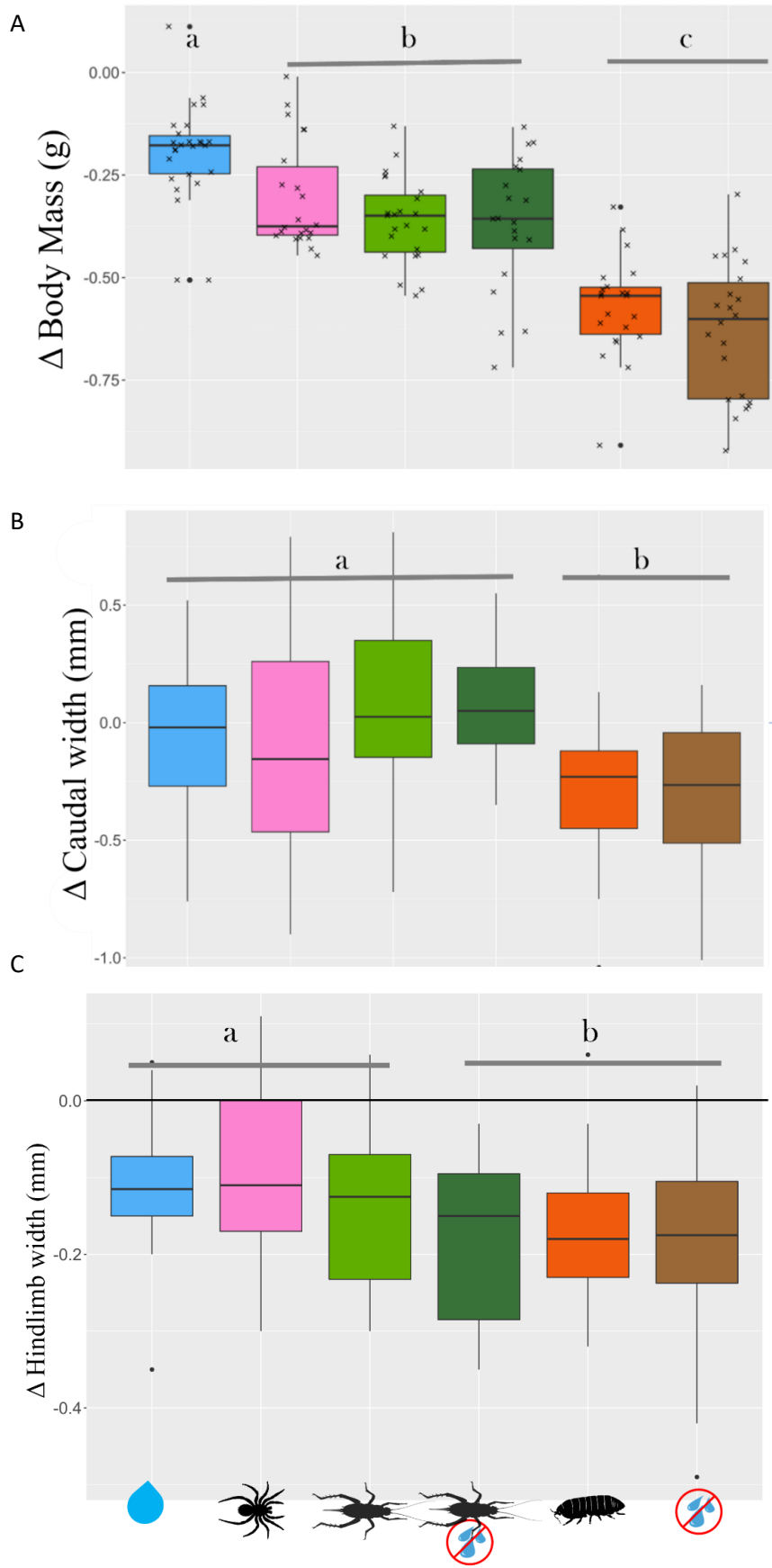


**Figure 1:** A) Timeline of the experimental design and of measurements performed between the start and the end of the manipulation in the different treatment groups. B) Boxplots of  $\Delta$ osmolality. Lower and upper hinges correspond to the first and third quartiles and middle bar is the median. Differences between groups are indicated based on *post hoc* tests with Tukey correction (from left to right: Hydrated control, Spiders, Hydrated crickets, Dehydrated crickets, Woodlice, and Dehydrated control). C) Scatterplot of  $\Delta$ osmolality against the amount of food consumed (g) for each treatment group with access to prey. Data shown are raw values and linear smooths for each group with a confidence interval in grey.

### 3.3. Plasma metabolites

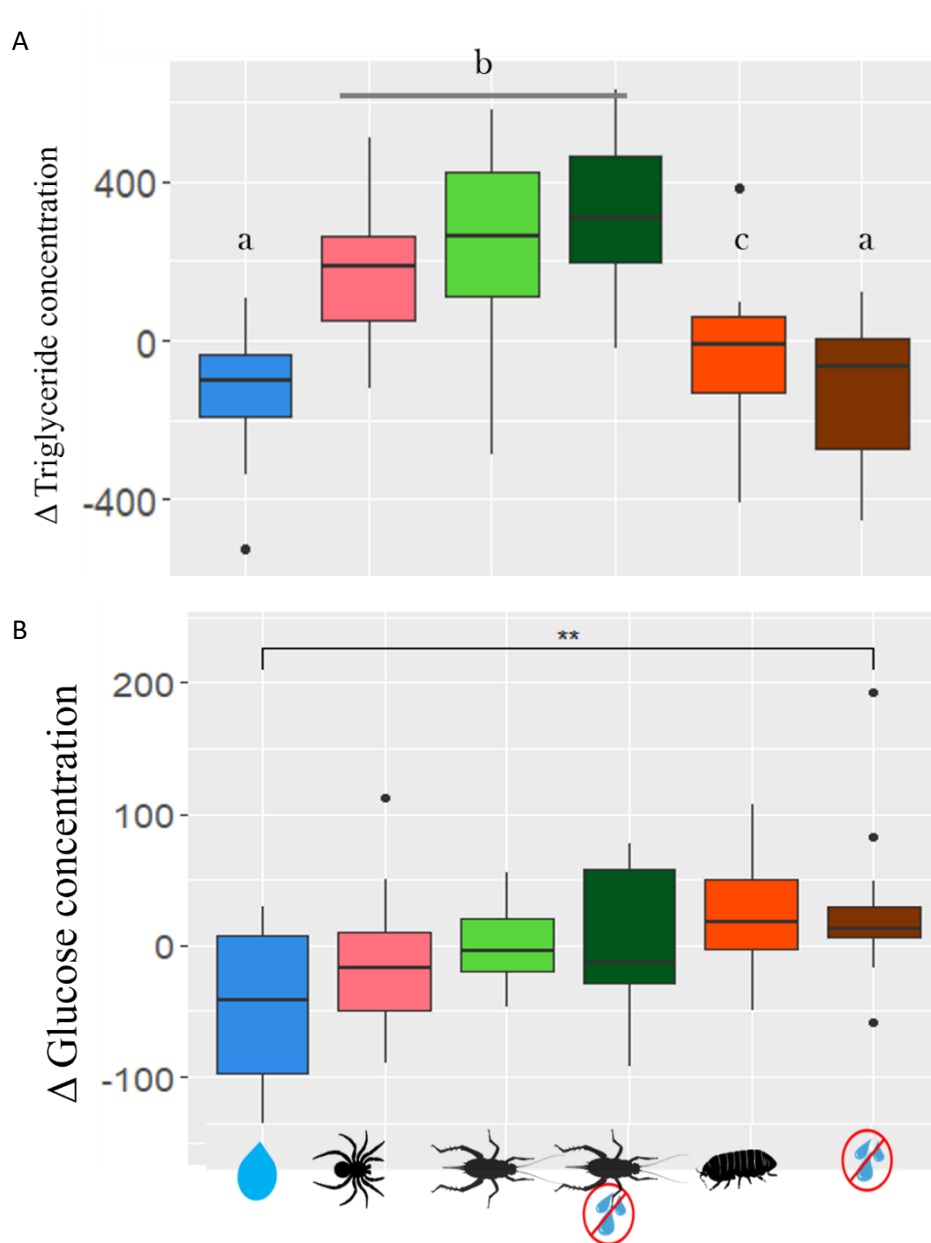
There was a strong effect of food treatment on triglyceride concentration changes in plasma ( $F_{5,88}=37.25$ ;  $p<0.0001$ ; Fig 3A), as well as an effect of initial concentration ( $F_{1,88} = 111.20$ ;  $p<0.0001$ ). *Post-hoc* analyses indicated three different treatment group categories. First, triglyceride concentrations on average decreased slightly in control lizards with no food irrespective of water availability. Second, triglyceride concentrations did not change on average in lizards provided with woodlice. Third, lizards fed with hydrated or dehydrated crickets and spiders had higher concentrations of plasma triglycerides (respectively, contrasts:  $\beta= 420.8$ ;  $\beta= 413.4$  and  $\beta= 286.5$ ; all  $p<0.0001$ , Fig 3A) compared to the other groups. Across all treatment groups with access to food, the amount of food consumed was positively correlated with the change in triglyceride concentrations ( $F_{1,53} = 7.83$ ;  $p=0.007$ ). At the same time, plasma  $\beta$ -HB concentrations slightly increased with a low variability among lizards and there was no significant difference among treatment groups ( $F_{5,118} = 1.44$ ,  $p=0.22$ ; initial concentration:  $F_{1,118} = 2.69$ ,  $p<0.0001$ ). Instead, intra-individual change of glucose concentration was highly variable and differed among treatment groups ( $F_{5,73} = 4.04$ ;  $p=0.0027$ ;  $F_{1,73} = 5.99$ ;  $p=0.017$ , Fig 3B). In the dehydrated control treatment, lizard glucose concentrations increased (estimate =  $67.83 \pm 18.94$ ;  $p=0.0006$ ) above the levels observed in the hydrated control treatment, whereas lizards provided with food had intermediate values. We found no effect of food consumption on glucose concentration changes ( $F_{1,45}=0.01$ ;  $p=0.92$ ).

**Figure 2:** Morphological changes A. Change in body mass (g, mean initial value:  $3,81 \pm 0,05$  g) between the start and the end of the manipulation in the different treatment groups. B Change in caudal width (mm, mean initial width  $6,46 \pm 0,04$ ) between the start and the end of the manipulation in the different treatment groups. C. Change in hindlimb width (mm, mean initial width  $1,98 \pm 0,01$ ) between the start and the end of the manipulation in the different treatment groups. Treatment groups are represented from left to right: Hydrated control, Spiders, Hydrated crickets, Dehydrated crickets, Woodlice, Dehydrated control. Lower and upper hinges correspond to the first and third quartiles and middle bar is the median. Differences between groups are indicated based on post hoc tests with Tukey correction.



**Figure 2**

**Figure 3:** Changes in blood parameters A. Change in triglyceride concentration in plasma between the start and the end of the manipulation in the different treatment groups. B. Change in glucose concentration in plasma between the start and the end of the manipulation in the different treatment groups. Treatment groups are represented from left to right: Hydrated control, Spiders, Hydrated crickets, Dehydrated crickets, Woodlice, Dehydrated control. Lower and upper hinges correspond to the first and third quartiles and middle bar is the median. Differences between groups are indicated based on post hoc tests with Tukey correction.





## 4. Discussion

In this study, we tested if prey consumption by a generalist mesopredator lizard could compensate for water deprivation via dietary water intake. Surprisingly, our results revealed that eating does not restore water balance, irrespective of prey type. On the contrary, osmolality increased similarly in all water-restricted lizards, with or without access to prey, whereas access to free-standing water was associated with restoration of an optimal hydration status. This result is remarkable given that non-fed animals lost about 0.8 g body mass during the experiment, which corresponds mostly to water loss through excretion in addition to respiratory and cutaneous evaporative water losses (Le Galliard *et al.*, 2021), whereas we provided 1.2 g of food with a mean water content of 72%, which is the equivalent of 0.85 g potential dietary water intake (Table 2). Thus, if lizards could extract significant amount of water from their food, they would have been able to compensate fully or partly for the loss of drinking water, especially when fed with higher quality prey types, but this was obviously not the case. In addition, our results suggest that the more the lizards eat, the more dehydrated they get, which would imply that food intake compromises water balance although this result must be interpreted with caution given that it is based on a correlation (Fig 1C). Altogether, these results imply that food consumption and digestion have a significant water cost whatever the prey type and that common lizards are therefore dependent on exogenous water intake (i.e., drinking) to maintain their water balance.

Only a few previous studies have investigated dietary water intake in squamate reptiles using experimental approaches. Direct tests on herbivorous and insectivorous species have found similar results, with food consumption reducing body mass loss due to water deprivation (Table 1, Munsey, 1972). However, osmolality was not measured, so previous knowledge of the effect on animal physiological hydration state is lacking. Studies focusing on carnivorous species explain the water costs of digestion through intermittent feeding and consumption of protein-rich prey (Wright *et al.*, 2013; Lillywhite, 2017). It is also possible that food consumption and metabolism, as well as the elimination of metabolic wastes, increase the requirement for water, potentially exacerbating dehydration during periods of drought (Lillywhite, 2017). In our study, lizards were insectivores and fed continuously, but increased dehydration due to feeding could be the result of an increased activity and body temperature due to the thermal and energetic requirements of foraging and digestion. These aspects may be of great importance in explaining the differences between non-fed and fed individuals, particularly in widely foraging and active lizards of small body size. Our results suggest that

lizards favored their energetic balance rather than their water balance, but we cannot determine if it was due to a digestion constraint or the result of a choice from woodlice-fed lizards to thermoregulate more, as we had no behavioral monitoring. If locomotion for foraging and thermoregulation to reach an optimal temperature for digestion are mostly responsible for increased water losses and negate possible dietary water gains, we expect these costs to be more significant in the field. Indeed, hunting has a much higher energetic and hydric cost in natural environments than in captivity (Anderson & Karasov, 1981). Therefore, complementary studies are needed to understand these digestion costs and the potential competition between feeding and hydration, which can sometimes lead to adaptation in metabolic rates (Gienger, Tracy, & Nagy, 2014).

Despite no evidence for the hypothesis that water can be extract from food from our data on plasma osmolality, a relevant metric of whole-organism hydration status (Capehart *et al.*, 2016), we found that food consumption can mitigate the structural and energetic costs due to chronic water deprivation, which included a general loss of body condition, muscle loss from the limb due to protein catabolism to release bound water (as shown in reptiles Bruschi *et al.*, 2018) and lipid catabolism from the reserves stored in the tail (Price, 2017). We chose three prey types corresponding to the natural diet of *Zootoca vivipara* (Vacheva & Naumov, 2019) and representing different trophic roles ranging from decomposers to insectivores (Bestion *et al.*, 2019) with distinct mobility strategies, morphology, as well as energy and nutrient content (Reeves *et al.*, 2021). In natural populations, common lizards forage actively on a wide diversity of invertebrate species including larvae and adult spiders, insects, isopods or gastropods (Vacheva & Naumov, 2019). For example, Avery (1966) found mostly spiders and homopters in stomach content of common lizards at two localities but also rarer preys such as ants and woodlice. Here, lizards provided with woodlice ate less readily during the experiment (on average 0.8 g instead of 1 g for the other prey types), possibly because these preys are less water and energy-rich (Table 2) and are less easy to handle and digest, making them distasteful for lizards (see Pekár, Líznavá, & Řezáč, 2016). As a consequence, the general body condition of woodlice-fed and water deprived lizards was much more impacted than in lizards fed with higher quality food such as crickets and spiders: these lizards lost more body mass, more muscle and more fat reserves from their tail, and were virtually indistinguishable from those held without food nor water. Thus, despite providing no significant net intake of water, high quality food and strong food consumption were important to recoup some of the energy costs of water deprivation and reduce the body condition loss induced by water stress. These results highlight

that feeding is important to maintain body condition, including muscular mass and reserves, which are important for future survival. Thus, while food may be important to cope with limited rainfall and water availability, it does not primarily provide lizards with water. Rather, it enables them to survive longer by maintaining a higher body condition, and improve their future prospects for reproduction by preserving muscles and reserves. Of course, this will require confirmation through more accurate assessments of the lizards' condition and improved tests with prey that are both hydrated and less hydrated since our manipulation of crickets did not yield reliable results in this regard (Table 2). Therefore, shifting diet toward higher water content prey would not allow this generalist lizard to avoid dehydration, but it can reduce the costs of drought on other physiological aspects of its organism (Dezetter *et al.*, 2022).

Unexpectedly, we found a significant difference between hydrated and dehydrated control groups in their plasma glucose concentration levels but no effect of food provisioning and water deprivation on plasma concentrations of ketone bodies ( $\beta$ -Hydroxybutyrate). Ketone bodies are usually synthesized in the fasting state from the catabolism of fatty acids or ketogenic amino acids and can be used as energy source by the brain when glucose levels are low (McCue *et al.*, 2012). It is predicted that their concentration should elevate during the second stage of fasting when carbohydrate stores are depleted and animals mobilize lipid stores instead (McCue *et al.*, 2012). However, previous studies have shown that plasma ketone concentrations do not necessarily increase during fasting in reptiles, especially when it is too short, and may even decrease when starvation is either extremely long (De Cássia Q. Pontes, Cartaxo, & Jonas, 1988). On the other side, the glucose levels in water-deprived lizards were maintained at relatively high levels, regardless of their feeding status. The experiment was conducted under temperature-controlled conditions to ensure that any differences observed were solely attributable to the variation in water availability or food type. Lizards with neither water nor food had much higher glucose concentration at the end of the manipulation than those from the hydrated control group. In vertebrates exposed to periods of fasting or intense exercise, maintenance of high glucose concentrations in the blood is ensured by gluconeogenesis in the liver (metabolic pathway that generates glucose from non-carbohydrate carbon substrates, Zain-ul-Abidin & Katorski, 1967) and glycogenolysis, the degradation of glycogen stored in adipose tissues or muscles (Storey, 1996). Gluconeogenesis involves glucose production in the liver from transported blood glycerol that is released by triglyceride hydrolysis in the tail or other reserve tissues. Glycerol levels and liver gluconeogenesis activity can increase during periods of fasting in snakes (Price, 2017). Thus, high glucose concentrations in the blood of fed and

water-deprived lizards may represent a higher capacity for gluconeogenesis due to lipid catabolism induced by fasting and dehydration. Similarly, water deprivation in the absence of food led to stronger muscle wasting, which may end up in muscle glycogen degradation and increased glucose production. This conversion of muscle stored glycogen into plasma circulating glucose was previously demonstrated in turtles (Emerson, 1967) and in amphibians and lizards after vigorous exercise, which is close to simulated conditions of fasting (Gleeson, 1982, 1991). However, our understanding of how stored macronutrients are utilized during fasting in reptiles remains limited compared to studies conducted on mammals and birds (Price, 2017). Our findings suggest that muscle breakdown (catabolism) in reptiles may commence earlier than expected in the starvation process, particularly in cases of water scarcity (Brusch *et al.*, 2018). Further physiological investigations would aid in elucidating these pathways and how they may be influenced by hydration status.

The effect of environmental temperatures on ectotherm metabolic rates is now well-studied (Schulte, 2015) but we know little about the other abiotic factors involved in the regulation of metabolism and energetic functions of the organisms. If digestion is strongly dependent on body temperature in reptiles for all diet (e.g. Harlow, Hillman, & Hoffman, 1976; Naulleau, 1983), how it is related to water balance and what is water availability impact on nutrient assimilation is poorly studied. When body temperature increases, evaporative water losses also increase, and it is accentuated by higher metabolism and respiration rates (Reilly, McBrayer, & Miles, 2007), which are closely linked to food intake. It seems then crucial to include food consumption and digestion to study trade-offs between temperature and water balance. We lack ambitious meta-analyses of reptile diet relative to climate at a global scale, but there seems to be a tendency towards herbivory in diurnal lizards (Meiri, 2018) and in warm and tropical zones (Zimmerman & Tracy, 1989) due to the physiological constraints imposed by herbivory, although it is not always verified (Espinoza, Wiens, & Tracy, 2004). However, various studies on ectotherms have demonstrated a shift toward herbivory with rising temperature in omnivorous species (Carreira *et al.*, 2016, 2017; Zhang *et al.*, 2020), which would certainly impact ecosystem functioning. Insectivorous and carnivorous reptiles also have their own physiological constraints, including trade-offs with water balance as we demonstrated here. This should be included in models trying to include water budget on species distribution under climate change (Urban *et al.*, 2016; Kearney *et al.*, 2018; Rubalcaba, Gouveia, & Olalla-Tárraga, 2019), as trade-offs between linked physiological traits are often essential to understand organism responses to environmental constraints (Riddell *et al.*, 2019) and it appears

here that the survival of water deprived animals could be highly dependent on food intake. Further studies would also allow us to have a deeper understanding of how digestive physiology constraints ecology on many aspects of an individual life history traits (Karasov & Diamond, 1988).

## 5. Acknowledgements

The authors want to thank Jean-Patrice Robin and the IPHC UMR 7178 for their help with the analysis of the invertebrate calorimetric contents. We also thank the intern students who help us with the experiment and animal care, especially Anna Marcout and Jules Domalain, as well as the CEREEP Ecotron IleDeFrance staff.

## 6. Competing interests

No competing interests declared.

## 7. Funding

This study received the financial and technical support from the Centre National de la Recherche Scientifique (CNRS) and was funded by the Agence Nationale de la Recherche AQUATHERM project (ANR-17-CE02-0013 to JFLG and OL).

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**Table 1:** Summary of empirical studies of dietary water intake in squamate reptiles.

| Species name                       | Standard diet | Climate zone | Link between diet and water balance  | Reference                            |
|------------------------------------|---------------|--------------|--|--------------------------------------|
| <i>Acrochordus granulatus</i>      | Carnivorous   | Marine       | Dietary water is insufficient for water balance  | Lillywhite, Heatwole, & Sheehy, 2014 |
| <i>Agkistrodon conanti</i>         | Carnivorous   | Semi-aquatic | Direct test. Food consumption increases post-prandial drinking in a laboratory experiment.   | Lillywhite, 2017                     |
| <i>Crotalus atrox</i>              | Carnivorous   | Semi-arid    | Direct test. Eating compromises water balance (osmolality) in chronic laboratory experiments of food consumption under water deprivation.  | Murphy & DeNardo, 2019               |
| <i>Heloderma suspectum</i>         | Carnivorous   | Xeric        | Direct test. Eating does not correct water balance (osmolality) in acute and chronic laboratory experiments of food consumption.   | Wright <i>et al.</i> , 2013          |
| <i>Lampropeltis californiae</i>    | Carnivorous   | Semi-arid    | Direct test. Food consumption increases post-prandial drinking in a laboratory experiment.   | Lillywhite, 2017                     |
| <i>Nerodia fasciata</i>            | Carnivorous   | Semi-aquatic | Direct test. Food consumption increases post-prandial drinking in a laboratory experiment.   | Lillywhite, 2017                     |
| <i>Nerodia clarkii</i>             | Carnivorous   | Marine       | Indirect test. Moderate dehydration with access to food (fish) inhibits feeding and leads to body mass loss.   | Edwards <i>et al.</i> , 2021         |
| <i>Nerodia fasciata</i>            | Carnivorous   | Semi-aquatic | Indirect test. Moderate dehydration with access to food (fish) inhibits feeding and leads to body mass loss.   | Edwards <i>et al.</i> , 2021         |
| <i>Pantherophis alleghaniensis</i> | Carnivorous   | Mesic        | Direct test. Food consumption increases post-prandial drinking in a laboratory experiment.   | Lillywhite, 2017                     |
| <i>Varanus rosenbergi</i>          | Carnivorous   | Mesic        | Indirect test. Field measures of water influx rates suggest that water is mostly provided from diet during the summer but not in other seasons. Free-standing water consumption unknown.                                       | Green, Dryden, & Dryden, 1991        |
| <i>Angolosaurus skoogi</i>         | Herbivorous   | Xeric        | Indirect test. Field measures of water influx rates suggest that water is mostly provided from a diet including succulent plants. Lizards apparently did not drink water but lost body mass.                                   | Nagy <i>et al.</i> , 1991            |
| <i>Dipsosaurus dorsalis</i>        | Herbivorous   | Xeric        | Indirect test. Field measures of water influx rates suggest that water is mostly provided from diet and lizards maintain a constant hydration state during the year, but shift to different plant materials during the summer. | Minnich & Shoemaker, 1970            |
| <i>Dipsosaurus dorsalis</i>        | Herbivorous   | Xeric        | Direct test. Food consumption allows to recoup part of the body mass loss induced by a chronic water deprivation experiment in the laboratory. Osmolality and body hydration was not measured.                                 | Munsey, 1972                         |

|                                |               |           |  |                                     |
|--------------------------------|---------------|-----------|--|-------------------------------------|
|                                |               |           |  |                                     |
| <i>Agama impalearis</i>        | Insectivorous | Xeric     | Indirect test. Field measures of water influx rates suggest that water is mostly provided from diet (termites) during breeding season. Lizards apparently did not drink water.   | Znari & Nagy, 1997                  |
| <i>Callisaurus draconoides</i> | Insectivorous | Semi-arid | Direct test. Food consumption allows to recoup the body mass loss induced by a chronic water deprivation experiment in the laboratory. Osmolality and body hydration was not measured.   | Munsey, 1972                        |
| <i>Cnemidophorus tigris</i>    | Insectivorous | Semi-arid | Direct test. Food consumption reduced the body mass loss induced by a chronic water deprivation experiment in the laboratory. Osmolality and body hydration was not measured.  | Munsey, 1972                        |
| <i>Sceloporus occidentalis</i> | Insectivorous | Mesic     | Direct test. Food consumption allows to recoup part of the body mass loss induced by a chronic water deprivation experiment in the laboratory. Osmolality and body hydration was not measured.   | Munsey, 1972                        |
| <i>Uma scoparia</i>            | Omnivorous    | Semi-arid | Indirect test. Field measures of water influx rates indicate stronger flux rates in juveniles feeding on insects than in adults feeding on dry plant materials. Lizards maintain water balance despite limited evidence that they drink free-standing water. | Minnich & Shoemaker, 1972           |
| <i>Uma scoparia</i>            | Omnivorous    | Semi-arid | Direct test. Food consumption (insects) allows to recoup part of the body mass loss induced by a chronic water deprivation experiment in the laboratory. Osmolality and body hydration was not measured.   | Munsey, 1972                        |
| <i>Varanus caudolineatus</i>   | Omnivorous    | Semi-Arid | Indirect test. Field and laboratory measures of water influx rates suggest that water is importantly provided from diet. Free-standing water consumption unknown.  | Thompson, Bradshaw, & Withers, 1997 |

## Supporting Information :

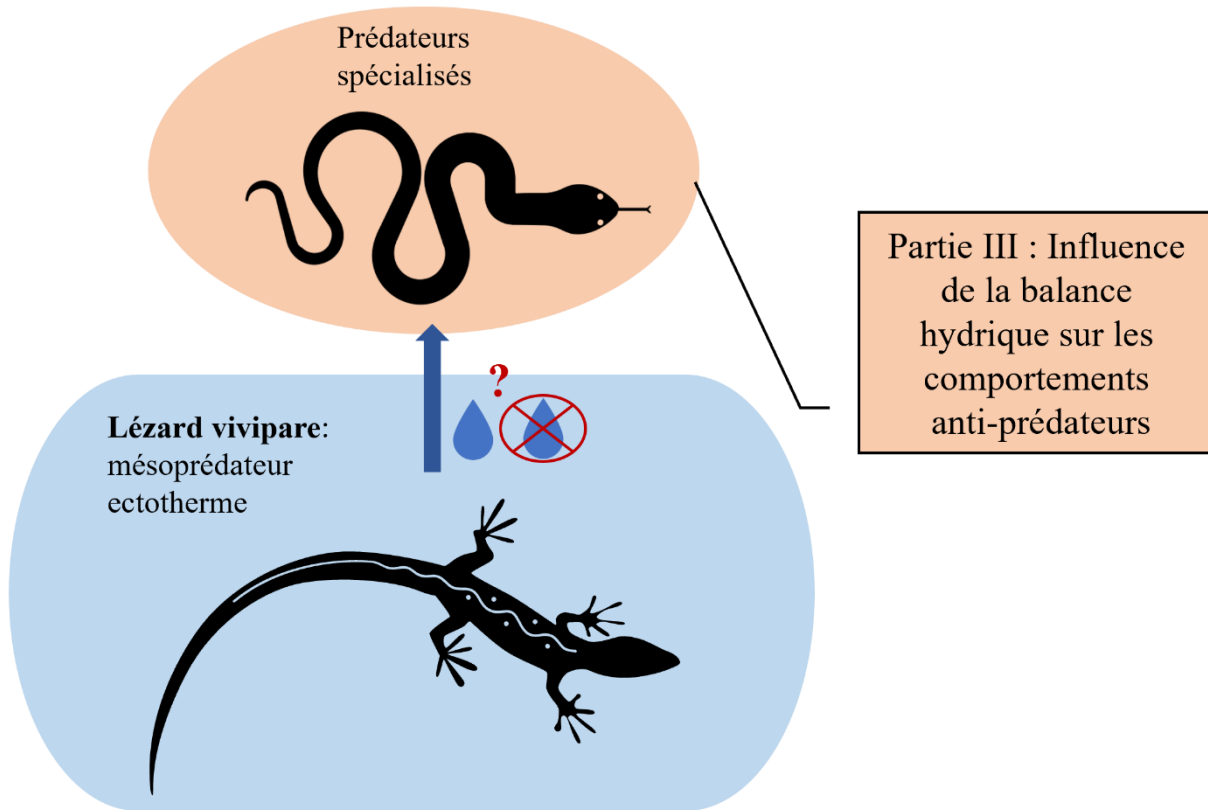
**Table S1** : Mean  $\pm$  SD morphological and physiological data for each treatment group over time

| Treatment                | Time | Body Mass        | Caudal width    | Muscle width    | Osmolality       | Triglycerides       | BHB (ketone)      | Glucose         |
|--------------------------|------|------------------|-----------------|-----------------|------------------|---------------------|-------------------|-----------------|
| Hydrated control N=22    | T0   | 3,875 $\pm$ 0,57 | 6,41 $\pm$ 0,54 | 1,98 $\pm$ 0,17 | 295,6 $\pm$ 42,9 | NA                  | NA                | NA              |
| Spiders N=21             | T0   | 3,701 $\pm$ 0,42 | 6,44 $\pm$ 0,58 | 1,98 $\pm$ 0,21 | 289,4 $\pm$ 38,3 | NA                  | NA                | NA              |
| Hydrated Crickets N=21   | T0   | 3,962 $\pm$ 0,43 | 6,64 $\pm$ 0,44 | 2,01 $\pm$ 0,13 | 284,6 $\pm$ 39,6 | NA                  | NA                | NA              |
| Dehydrated Crickets N=22 | T0   | 3,743 $\pm$ 0,55 | 6,54 $\pm$ 0,51 | 1,97 $\pm$ 0,18 | 298,4 $\pm$ 26,3 | NA                  | NA                | NA              |
| Woodlice N=21            | T0   | 3,802 $\pm$ 0,65 | 6,38 $\pm$ 0,52 | 1,94 $\pm$ 0,15 | 286,7 $\pm$ 47,3 | NA                  | NA                | NA              |
| Dehydrated control N=22  | T0   | 3,785 $\pm$ 0,58 | 6,39 $\pm$ 0,47 | 1,98 $\pm$ 0,14 | 292,3 $\pm$ 34,8 | NA                  | NA                | NA              |
|                          |      |                  |                 |                 |                  |                     |                   |                 |
| Treatment                | Time | Body Mass        | Caudal width    | Muscle width    | Osmolality       | Triglycerides       | BHB (ketone)      | Glucose         |
| Hydrated control N=22    | T1   | 3,959 $\pm$ 0,59 | 6,40 $\pm$ 0,50 | 1,96 $\pm$ 0,13 | 327,5 $\pm$ 28,5 | 241,76 $\pm$ 196,40 | 0,507 $\pm$ 0,177 | 320,5 $\pm$ 137 |
| Spiders N=21             | T1   | 3,735 $\pm$ 0,39 | 6,38 $\pm$ 0,50 | 1,93 $\pm$ 0,14 | 337,2 $\pm$ 35,6 | 242,17 $\pm$ 123,26 | 0,502 $\pm$ 0,161 | 273,4 $\pm$ 93  |
| Hydrated Crickets N=21   | T1   | 3,970 $\pm$ 0,43 | 6,59 $\pm$ 0,41 | 2,00 $\pm$ 0,13 | 338,5 $\pm$ 33,8 | 277,30 $\pm$ 167,41 | 0,529 $\pm$ 0,170 | 275,6 $\pm$ 98  |
| Dehydrated Crickets N=20 | T1   | 3,804 $\pm$ 0,66 | 6,36 $\pm$ 0,44 | 1,96 $\pm$ 0,17 | 335,8 $\pm$ 30,7 | 209,25 $\pm$ 84,83  | 0,531 $\pm$ 0,230 | 309,8 $\pm$ 111 |
| Woodlice N=21            | T1   | 3,865 $\pm$ 0,54 | 6,41 $\pm$ 0,43 | 1,97 $\pm$ 0,15 | 333,4 $\pm$ 33,5 | 271,49 $\pm$ 179,71 | 0,656 $\pm$ 0,310 | 250,3 $\pm$ 75  |
| Dehydrated control N=22  | T1   | 3,948 $\pm$ 0,59 | 6,49 $\pm$ 0,42 | 2,00 $\pm$ 0,16 | 324,6 $\pm$ 35,7 | 262,09 $\pm$ 147,32 | 0,496 $\pm$ 0,133 | 233,4 $\pm$ 84  |
|                          |      |                  |                 |                 |                  |                     |                   |                 |
| Treatment                | Time | Body Mass        | Caudal width    | Muscle width    | Osmolality       | Triglycerides       | BHB (ketone)      | Glucose         |
| Hydrated control N=22    | T2   | 3,568 $\pm$ 0,54 | 6,35 $\pm$ 0,44 | 1,84 $\pm$ 0,13 | 316,9 $\pm$ 24,2 | 107,54 $\pm$ 62,90  | 0,716 $\pm$ 0,304 | 232,9 $\pm$ 101 |
| Spiders N=21             | T2   | 3,527 $\pm$ 0,47 | 6,23 $\pm$ 0,57 | 1,82 $\pm$ 0,16 | 368,5 $\pm$ 36,3 | 625,01 $\pm$ 365,72 | 0,682 $\pm$ 0,381 | 293,0 $\pm$ 104 |
| Hydrated Crickets N=20   | T2   | 3,788 $\pm$ 0,41 | 6,64 $\pm$ 0,43 | 1,88 $\pm$ 0,13 | 365,2 $\pm$ 32,8 | 639,69 $\pm$ 291,70 | 0,607 $\pm$ 0,136 | 292,9 $\pm$ 117 |
| Dehydrated Crickets N=19 | T2   | 3,628 $\pm$ 0,56 | 6,44 $\pm$ 0,53 | 1,80 $\pm$ 0,14 | 371,1 $\pm$ 36,1 | 679,28 $\pm$ 382,99 | 0,798 $\pm$ 0,256 | 318,3 $\pm$ 111 |
| Woodlice N=21            | T2   | 3,201 $\pm$ 0,49 | 6,14 $\pm$ 0,46 | 1,79 $\pm$ 0,11 | 367,9 $\pm$ 24,8 | 222,73 $\pm$ 149,52 | 0,803 $\pm$ 0,242 | 293,3 $\pm$ 100 |
| Dehydrated control N=22  | T2   | 3,150 $\pm$ 0,51 | 6,18 $\pm$ 0,52 | 1,81 $\pm$ 0,12 | 358,2 $\pm$ 30,5 | 163,67 $\pm$ 95,91  | 0,743 $\pm$ 0,311 | 286,9 $\pm$ 121 |

**Table S2** : Summary of best models explaining variation of our morphological and physiological variables (reference Lot = Hydrated control)

| Variable                           | Explanatory variables (and factors) | Estimate    | SE         | t value      | P value      |     |
|------------------------------------|-------------------------------------|-------------|------------|--------------|--------------|-----|
| Δ Osmo                             | Intercept                           | -10.78261   | 5.608304   | -1.922615    | 5.685951e-02 | *   |
|                                    | Lot (Spiders)                       | 46.11594    | 8.117985   | 5.680713     | 9.288206e-08 | *** |
|                                    | Lot (Hydrated Crickets)             | 43.60079    | 8.020962   | 5.435855     | 2.842758e-07 | *** |
|                                    | Lot (Dehydrated Crickets)           | 48.15103    | 8.338336   | 5.774657     | 6.005258e-08 | *** |
|                                    | Lot (Woodlice)                      | 46.87352    | 8.020962   | 5.843877     | 4.344574e-08 | *** |
|                                    | Lot (Dehydrated control)            | 44.97308    | 8.117985   | 5.539932     | 1.772812e-07 | *** |
| Δ Osmo                             | (Intercept)                         | -0.4799205  | 17.671406  | -0.02715802  | 0.97840218   |     |
|                                    | Lot (Spiders)                       | 1.1319035   | 7.758376   | 0.14589439   | 0.88437639   |     |
|                                    | Lot (Dehydrated Crickets)           | 3.2593316   | 7.958940   | 0.40951831   | 0.68326760   |     |
|                                    | Lot (Woodlice)                      | 9.7628505   | 8.310900   | 1.17470433   | 0.24364174   |     |
|                                    | food consumed                       | 34.2733345  | 17.319002  | 1.97894397   | 0.05130687   | *   |
| Δ Body Mass                        | Intercept                           | -0.04173397 | 0.09119913 | -0.4576136   | 0.6480       |     |
|                                    | Lot (Spiders)                       | -0.11535743 | 0.03935334 | -2.9313248   | 0.0040       | **  |
|                                    | Lot (Hydrated Crickets)             | -0.16018805 | 0.03870043 | -4.1391804   | 6.276944e-05 | *** |
|                                    | Lot (Dehydrated Crickets)           | -0.17308132 | 0.04018403 | -4.3072168   | 3.262985e-05 | *** |
|                                    | Lot (Woodlice)                      | -0.37875057 | 0.03918139 | -9.6665932   | 6.238080e-17 | *** |
|                                    | Lot (Dehydrated control)            | -0.43480937 | 0.03916402 | -11.1022655  | 1.787490e-20 | *** |
|                                    | Initial body mass                   | -0.03995397 | 0.02249626 | -1.7760268   | 0.0781       | .   |
| Δ Caudal Width                     | Intercept                           | -0.39569440 | 0.23489339 | -1.6845702   | 0.094628148  |     |
|                                    | Lot (Spiders)                       | -0.05748813 | 0.10306791 | -0.5577694   | 0.578023909  |     |
|                                    | Lot (Hydrated Crickets)             | 0.10170261  | 0.10293750 | 0.9880035    | 0.325106372  |     |
|                                    | Lot (Dehydrated Crickets)           | 0.10825851  | 0.10426998 | 1.0382520    | 0.301206121  |     |
|                                    | Lot (Woodlice)                      | -0.24125508 | 0.10017493 | -2.4083380   | 0.017521669  | **  |
|                                    | Lot (Dehydrated control)            | -0.26248017 | 0.10012980 | -2.6213991   | 0.009871372  | *** |
| Δ Hindlimb Width                   | Intercept                           | 0.09198579  | 0.05798077 | 1.5864880    | 0.115217207  |     |
|                                    | Intercept                           | -0.123269   | 0.019937   | -6.183       | 7.94e-09     | *** |
|                                    | Lot (Spiders)                       | 0.009178    | 0.029449   | 0.312        | 0.7558       |     |
|                                    | Lot (Hydrated Crickets)             | -0.009231   | 0.029449   | -0.313       | 0.7545       |     |
|                                    | Lot (Dehydrated Crickets)           | -0.035941   | 0.030682   | -1.171       | 0.2436       |     |
|                                    | Lot (Woodlice)                      | -0.059913   | 0.029449   | -2.034       | 0.0440       | *   |
| Δ Triglycerides                    | Lot (Dehydrated control)            | -0.064003   | 0.029449   | -2.173       | 0.0316       | *   |
|                                    | Intercept                           | 73.05162    | 36.28466   | 2.013        | 0.04714      | *   |
|                                    | Lot (Spiders)                       | 295.45556   | 46.91350   | 6.298        | 1.15e-08     | *** |
|                                    | Lot (Hydrated Crickets)             | 429.68976   | 46.59329   | 9.222        | 1.43e-14     | *** |
|                                    | Lot (Dehydrated Crickets)           | 421.22149   | 48.21797   | 8.736        | 1.44e-13     | *** |
|                                    | Lot (Woodlice)                      | 125.30000   | 42.94764   | 2.918        | 0.00448      | *** |
|                                    | Lot (Dehydrated control)            | 45.82324    | 42.96092   | 1.067        | 0.28906      |     |
| Initial triglyceride concentration | -0.88742                            | 0.08415     | -10.545    | 2.753698e-17 | ***          |     |
| Δ Triglycerides                    | (Intercept)                         | -318.37     | 202.11     | -1.575       | 0.12115      |     |
|                                    | Lot (Spiders)                       | -51.13      | 76.90      | -0.665       | 0.50897      |     |
|                                    | Lot (Dehydrated Crickets)           | 93.58       | 78.58      | 1.191        | 0.23900      |     |
|                                    | Lot (Woodlice)                      | -118.92     | 84.33      | -1.410       | 0.16434      |     |
|                                    | food consumed                       | 535.20      | 191.22     | 2.799        | 0.00714      | *** |
| Δ Ketone bodies (BHB)              | Intercept                           | 0.53456     | 0.08779    | 6.089        | 1.46e-08     | *** |
|                                    | Lot (Spiders)                       | -0.03058    | 0.08269    | -0.370       | 0.712        |     |
|                                    | Lot (Hydrated Crickets)             | -0.10753    | 0.08378    | -1.283       | 0.202        |     |
|                                    | Lot (Dehydrated Crickets)           | 0.08871     | 0.08628    | 1.028        | 0.306        |     |
|                                    | Lot (Woodlice)                      | 0.01324     | 0.08511    | 0.156        | 0.877        |     |
|                                    | Lot (Dehydrated control)            | 0.03601     | 0.08269    | 0.435        | 0.664        |     |
|                                    | Initial BHB concentration           | -0.64833    | 0.13322    | -4.867       | 3.55e-06     | *** |
| Δ Glucose                          | Intercept                           | -4.63185    | 21.48980   | -0.216       | 0.829950     |     |
|                                    | Lot (Spiders)                       | 33.85835    | 20.14297   | 1.681        | 0.097056     |     |
|                                    | Lot (Hydrated Crickets)             | 45.15655    | 19.20440   | 2.351        | 0.021406     | *   |
|                                    | Lot (Dehydrated Crickets)           | 57.49766    | 19.43093   | 2.959        | 0.004158     | **  |
|                                    | Lot (Woodlice)                      | 65.68479    | 19.27285   | 3.408        | 0.001068     | **  |
|                                    | Lot (Dehydrated control)            | 67.83226    | 18.93878   | 3.582        | 0.000612     | *** |
|                                    | Initial glucose concentration       | -0.15337    | 0.06263    | -2.449       | 0.016737     | *   |

Partie III : Influence de la balance hydrique sur les comportements anti-prédateurs





## Résumé :

Cette troisième partie s'intéresse au lézard vivipare en tant que proie, afin d'étudier comment son état hydrique individuel peut influencer sa réponse aux prédateurs. Elle comprend deux chapitres, dont l'un est accepté pour publication et l'autre en préparation.

Le premier chapitre présente une étude comportementale sur les interactions entre deux contraintes environnementales : la disponibilité en eau et la présence de prédateurs, et leurs impacts sur les stratégies de thermo-hydrorégulation du lézard vivipare. Nous avons analysé les changements de comportement de thermo-hydrorégulation, les patterns d'activité et la température corporelle en réponse à un stress hydrique chronique couplé à une présence d'odeurs de prédateurs. La restriction hydrique et la présence de prédateurs ont principalement des effets additifs sur le comportement de thermorégulation du lézard vivipare. Ils réduisent notamment le temps passé à se chauffer et la précision de la thermorégulation. Ils ont en revanche des effets opposés sur le temps d'activité, la restriction hydrique réduisant l'activité tandis que la présence d'odeurs de prédateurs l'augmente. Nous avons également constaté un effet interactif sur un comportement d'hydrorégulation, les lézards soumis à une restriction hydrique montrant une préférence pour les abris humides (leur permettant d'hydroréguler) seulement en l'absence d'odeur de prédateurs. Cette étude démontre que les réponses comportementales face à la menace de prédation peuvent dépendre de l'état d'hydratation des individus, suggérant que la peur des prédateurs peut compromettre la thermo-hydrorégulation et donc les performances des proies.

Le deuxième chapitre examine les coûts hydrique et énergétique de la chémoréception chez le lézard vivipare, un sens qui leur permet de détecter et de reconnaître leurs prédateurs. Ces coûts sont évalués à l'aide de mesures de respirométrie, où les lézards sont exposés à des odeurs de prédateurs pendant la mesure des échanges de gaz et d'eau avec l'air environnant. Une expérience supplémentaire vient compléter l'étude, au cours de laquelle les lézards subissent une déshydratation et nous mesurons leurs comportements de détection des prédateurs (notamment le *tongue flick rate*, TF) afin de déterminer si la chémoréception est impactée par la déshydratation. Les résultats indiquent que l'exposition aux odeurs de prédateurs entraîne une augmentation des TF, ce qui se traduit par une dépense énergétique accrue, révélant ainsi un coût énergétique associé à la chémoréception. En revanche, le nombre de TF n'a pas d'effet direct sur les pertes hydriques évaporatives, même si la présence d'odeurs de prédateurs augmente les pertes hydriques totales, suggérant des changements comportementaux et physiologiques avec un coût hydrique. Par ailleurs, les lézards déshydratés réduisent fortement leurs TF et perdent leur capacité à reconnaître des odeurs de prédateurs, ce qui affecte d'autres comportements anti-prédateurs comme la fuite. Cette étude met en évidence l'importance de la balance hydrique pour la chémoréception et l'évitement des prédateurs chez les lézards, révélant un compromis entre le maintien de la balance hydrique et une réponse efficace à la présence de prédateurs.

## **Chapitre 5) Behavioural response to predation risks depends on experimental change in dehydration state in a lizard**

*Accepted for publication in Behavioural Ecology and Sociobiology*

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Running title: Behavioural responses to water and predation stresses combined

## **Abstract**

Optimal regulation of body temperature and water balance is essential for the survival of terrestrial ectotherms in a changing world. A behavioural trade-off exists between these two constraints because maintaining a high body temperature usually increases evaporative water losses. In addition, the evaluation of predation risk is a key factor in behavioural decision for prey species, and predation threat can cause shift in individual behaviours due to the modification the cost-benefit balance of thermo-hydroregulation. However, little is known on how preys integrate these different biotic and abiotic stressors when combined. Here, we performed an experimental study on the common lizard, a terrestrial ectotherm prey species, sensitive to water restriction and able to detect specialized predator scents in its environment. We analysed changes in thermo-hydroregulation behaviours, activity patterns and body temperature in response to a chronic water stress coupled with simulated punctual occurrences of predator scents. Water restriction and predator threat had mostly additive effects on lizard thermoregulation behaviour. They both reduced the time spent basking and thermoregulation precision. They also had opposite effects on the time spent active, water restriction reducing activity whereas the presence of predator scents increased it. Yet, we also found an interactive effect on hydroregulation behaviour, as water restricted lizards showed a wet-shelter preference only in absence of predator odours. This study demonstrates the existence of some hydration state dependent behavioural responses to predator threat and suggests that fear of predators may compromise thermo-hydroregulation and thus prey performances.

Keywords: thermoregulation - lizards – dehydration – predation risk – reptiles – activity pattern

## 1. Introduction

Water is one of the most essential resource for the survival and reproduction of organisms, and natural selection has led to several behavioural adaptations and acclimation strategies enabling terrestrial organisms to cope with predictable and unpredictable reductions of water availability (Davies, 1982; Chown, Sørensen & Terblanche, 2011; Pirtle, Tracy & Kearney, 2019; Fuller *et al.*, 2021). In ectotherms, thermoregulation is primarily behavioural (Angilletta Jr, 2009) and there is a potential conflict between water balance and body temperature regulation because thermoregulation effort and high body temperatures generally increase evaporative water loss through the skin and during respiration (e.g., Lourdais *et al.*, 2017). Thus, water constraints influence thermoregulation precision and dehydration should lead to “sub-optimal” body temperature in terrestrial ectotherms (Huey & Slatkin, 1976; Anderson & Andrade, 2017; Rozen-Rechels *et al.*, 2019). Indeed, there is now accumulating evidence that dehydration is associated with lower field body temperatures during activity (Ladyman & Bradshaw, 2003), reduction in preferred body temperatures in the laboratory (Sannolo & Carretero, 2019), decrease of behavioural activity or shifts in the daily and seasonal activity patterns (Rozen-Rechels *et al.*, 2020). This surge of interest for the understanding of the joint mechanisms involved in the regulation of body temperature and water balance led to the development of the thermo-hydroregulation concept (Rozen-Rechels *et al.*, 2019), defined as the integrated suite of behavioural and physiological processes enabling homeostatic regulation of both body temperature and hydration state.

Biotic interactions such as predation are also a major selective force shaping behavioural strategies. The detection of specialized predators in the habitat can induce significant behavioural changes in their prey (Clinchy, Sheriff & Zanette, 2013). Anti-predator behavioural responses include temporal and spatial shifts in activity, changes in dispersal behaviour or differential investment in vigilance effort of the prey (Brown, Laundre & Gurung, 1999; Martin, 2011). However, to date, little is known about the combined influences of dehydration and predation risks on thermo-hydroregulation behaviours and whether these two stressors have additive or interactive effects on thermoregulation. Yet, this is highly relevant to patterns and processes of thermo-hydroregulation because these two constraints should influence jointly the non-energetic costs of thermo-hydroregulation behaviours and these effects should be driven by their spatiotemporal variations in natural populations. For example, simultaneously avoiding predation and fulfilling thermo-hydroregulation needs may be in conflict, as shown in recent studies of ecological interactions between ungulates and their predators in semi-arid areas, such

as African savannahs (Veldhuis *et al.*, 2019). In these habitats, large predators are attracted by waterholes such that water dependent ungulate species also experience a higher exposure to predation risks, leading to temporal or spatial shifts in their thermo-hydroregulation strategies (Valeix *et al.*, 2009b). Another possibility includes shifts in social behaviour in response to water deprivation that increase the risks of predation, such as huddling behaviours in amphibians (Rohr & Madison, 2003). In such situations, the benefits of thermo-hydroregulation responses to water deprivation may be cancelled by a simultaneous increase in predation risks (Rohr & Madison, 2003).

In addition to interactions between predation risks and water availability caused by their level of complementation in the landscape, preys may react to predation risks differently depending on their hydration state. This is especially likely in organisms that can tolerate a wide range of osmotic states and display large variations in their hydration status, such as many terrestrial ectotherms (Lillywhite, 2016). According to classical behavioural models (Lima, 1998), if a trade-off exists between avoiding predators and getting necessary water resources, dehydrated individuals should be more prone to take risks in the presence of predators than well-hydrated individuals. Alternatively, since the dehydration state of the individual also influences the potential costs and benefits of thermoregulation in a risky environment (Rozen-Rechels *et al.*, 2019), anti-predator responses may involve changes in thermoregulation, such as reduction in behavioural activity or changes in micro-habitat selection. Changes in body condition induced by chronic dehydration could also influence an individual's response to the fear of a predator, which is generally condition-dependent (e.g. in lizards, Martín & López, 1999; Martín, López & Cooper Jr, 2003).

In terrestrial ectotherms, thermoregulation plays an important role in predator avoidance because high locomotor performances allowing prey to escape from their predators are closely related to an optimal regulation of body temperature (Landry Yuan *et al.*, 2021). However, behavioural thermoregulation often involves increased activity (i.e., shuttling behaviours) and preferential use of open habitats (i.e., basking behaviours), which can draw the attention of predators and increase predation risks. Therefore, there is a proximate trade-off between active thermoregulation strategies and the exposure to predation (Angilletta Jr, 2009). For example, terrestrial ectotherms can adjust their escape tactics and flight initiation distances in relation to their body temperature and opportunities for optimal basking in their environment (Cooper, 2009). Furthermore, reptiles often use burrows or crevices as shelters to protect themselves from predators and they will use differentially these shelters depending on their thermal quality

and their perception of predation risks (Amo, López & Martín, 2004; Lorigoux, Lisse & Lourdais, 2013). Generally, the perception of predators triggers a decrease in behavioural activity and may reduce the accuracy of thermoregulation (Downes, 2001; Herczeg *et al.*, 2008; Angilletta Jr, 2009; Lorigoux *et al.*, 2013). These organisms thus provide relevant models to address interaction between predation risks, thermoregulation and water constraints, even though this facet has been poorly studied.

Here, we set up a laboratory experiment in a ground-dwelling lizard (the common lizard, *Zootoca vivipara*) to quantify changes in thermo-hydroregulation behaviours when animals are exposed to simultaneous changes in dehydration risks and the threat of predation. Common lizards are widespread lizards from cold and wet habitats across Eurasia and they are preyed upon by a diversity of generalist and specialist predators, including snakes such as the adder (Prestit, 1971) and the smooth snake (Drobenkov, 2014). Previous studies have demonstrated that there is an overall reduction in thermoregulation effort for animals under water stress (Rozen-Rechels *et al.*, 2020). Common lizards and their predators rely on chemical cues in their environment to detect each other, and previous studies have shown that common lizard can recognize scent from a specialized snake predator and differentiate it from the scent of food or of conspecifics (Thoen, Bauwens & Verheyen, 1986; Damme *et al.*, 1990). Here, we examined the following hypothesis. First, we expect a reduction in the proportion of time spent basking and the activity window (and thus being more vulnerable to predators) for individuals confronted to scents of their predators. Lizards could also lower their body temperature (Martin & Huey, 2008; Anderson & Andrade, 2017) and shift toward more thermo-conformity under stressful conditions as predicted by Huey & Slatkin, 1976. We also expect adult males to be more prone to take risks than females, as it has been suggested in this species by Antczak *et al.*, 2019. Second, we posit that lizards confronted to predator scents in addition to water stress will respond differently than well-hydrated lizards, as antipredator behaviours are often condition dependent. Dehydrated lizards will need to take more risk to compensate for the lack of water and then decrease basking behaviour and the time of day at thermal preference, even if this impacts their ability to escape predators.

To test these hypotheses, we submitted lizards to two different conditions including a control with access to *ad libitum* drinking water and a treatment group with restricted access to drinking water, hereafter referred to as the water treatment. Before and after this manipulation, we recorded lizards' behaviours and activity, as well as their body temperature, under two

conditions of predator threat (absence or presence of predator scents in the environment). This experimental design allows us to detect a potential interactive effect between the two stressors.

## **2. Material and methods**

### 2.1. Studied species and acclimation conditions

The common lizard *Zootoca vivipara* is a small lacertid lizard (Reptilia: Lacertidae; adult snout-vent length 50-70mm) with a wide Euro Siberian distribution (Surget-Groba *et al.*, 2006). It inhabits mesic environments such as humid grasslands and peat bogs and is highly sensitive to water deprivation due to its high standard water loss rates (Lorenzon *et al.*, 1999; Massot *et al.*, 2002; Dupoué *et al.*, 2017). The species is mainly predated by birds and snakes including the adder (Viperidae, *Vipera berus*) and several species of Colubridae (Prestit, 1971; Steen, Løw & Sonerud, 2011; Drobenkov, 2014). Common lizards used in this study were captured after the reproduction period (in early July 2019) in semi-natural populations maintained in 10 fenced, outdoor enclosures in CEREEP-Ecotron IleDeFrance in Saint Pierre lès Nemours, France (48°17'N, 2°41'E) without natural predators. We captured 72 adults ( $\geq 2$  years old) with a 1:1 sex ratio, measured their snout-vent length with a plastic ruler ( $\pm 0.5$ mm), and weighted them for body mass ( $\pm 1$ mg). Lizards were then placed in individual terrarium (18  $\times$  11  $\times$  12cm) for acclimation in a temperature-controlled room (23°C from 8:00 to 19:00, 15°C otherwise) and fed with living crickets (*Acheta domestica*) *ad libitum*. During acclimation, drinking water was available *ad libitum* in a water cup and terraria were sprayed 3 times a day with water to maintain a wet environment.

### 2.2 Experimental design

After a one-week acclimation period, lizards were divided into 3 trial groups (24 individuals per group, 12 females and 12 males) tested successively. Inside each group, individuals were paired in couple of one female and one male so that their body sizes match and behavioural observations were recorded on pairs of individuals. The day before the first behavioural observations, each pair was transferred to a neutral arena (79  $\times$  57  $\times$  42cm) in a temperature-controlled room maintained at 25°C between 8:00 am and 5:30 pm. Arena were equipped with a substratum of sterilized peat soil, a water cup and, on one side, two 40W light bulbs placed above two artificial shelters in order to provide them with basking spots and refuges in the so-called “hot zone” of the arena and a retreat site without refuge in the “cold part” of the arena. One shelter was maintained humid at ground level with a wet sponge, whereas the other one was maintained dry with a dry sponge. In addition, a UV neon tube (Reptisun 10.0, white light)

provided UV enhanced light above each arena during daytime. Mean temperature in wet shelter was slightly lower than in dry shelter (wet: 27.1°C; dry: 27.9°C), and mean relative humidity was strongly higher in the wet one (wet: 54.4%; dry: 35.4%). On the cold side of the arena (without the heating lamps above), ground temperatures stayed below *Zootoca vivipara* preferred temperature all day long whereas the hot part offered optimal temperatures for thermoregulation (temperature during behavioural trial: cold mean = 26.5°C, maximum = 28.5°C ; hot mean = 35.4°C, maximum = 39.8°C, Gvoždík & Castilla, 2001).

The experimental design consisted in 2 days of behavioural observations prior to manipulation, 8 days of manipulation of water availability, and 2 days of observations after this manipulation period (see Figure 1). First, behavioural observations (see below) were performed with lizards maintained in standard conditions (including *ad libitum* water) for each pair with a random succession of each of the two predator trial groups (“control” trial group with no scent or “odor” trial group with predator scents added in the arena) during one day each. We referred to this sequential test as the “test day sequence” variable and our sequential test was motivated by the need to avoid habituation of lizards to scents since they were tested against predator scents only during one day (Parsons *et al.*, 2018). The odor trial group was obtained by combining scents of two natural predators of the common lizards. We used a standardized quantity of shed skins of the adder (*Vipera berus*) melt with the substrate layer in addition to shelters installed in a live smooth snake’s (*Coronella austriaca*) terrarium for at least 24h, in order to imitate a realist situation as these two predators can be sympatric. Shed skins were obtained from adders (N = 40) captured in Western France and temporarily maintained in captivity at CEBC, CNRS (Dezetter *et al.*, 2021). The smooth snakes (N = 2) were captured in natural habitats at CEREEP-Ecotron in late June 2019. Next, after these two days, lizards were put back in their individual terrarium for 8 days without odors of predators. Water treatment (control *ad libitum* or restricted water) was randomly attributed to each pair following standard protocols: in control conditions, each terrarium had a water cup filled with drinking water in the morning whereas we removed the water in restricted conditions (Dupoué *et al.*, 2018). In addition, we reduced water spray to once every day in restricted conditions instead of 3 times a day in control conditions. Lizards were fed with living cricket *Acheta domestica* throughout the experiment. Finally, after these 8 days manipulation period, pairs of lizards were placed back in the neutral arena for 2 additional days of behavioural observations involving a new random sequence of each of the two predator trial groups (see Figure 1).



| Behavioural trials (2 days)   | Water manipulation (8 days) | Behavioural trials (2 days)      |
|---|-----------------------------|----------------------------------|
| Presence of predator scents N=72<br>Absence of predator scents N=72 | Water control group N=36    | Presence of predator scents N=36 |
|   |                             | Absence of predator scents N=36  |
|   | Water restricted group N=36 | Presence of predator scents N=36 |
|   |                             | Absence of predator scents N=36  |
| <i>Neutral arena</i>  | <i>Individual terrarium</i> | <i>Neutral arena</i>             |

|  |   |  |
|--|---|--|
| <p>■ Measurements:<br/> (1) <i>Active or not</i><br/> (2) <i>Behaviour</i><br/> (3) <i>Zone</i><br/> (4) <i>Body temperature</i></p> | <p>■ Measurements before and after:<br/> (1) <i>Body mass</i><br/> (2) <i>Plasma osmolality</i></p> | <p>■ Measurements:<br/> (1) <i>Active or not</i><br/> (2) <i>Behaviour</i><br/> (3) <i>Zone</i><br/> (4) <i>Body temperature</i></p> |
|--|---|--|

**Fig.1** Timeline of the experimental design including behavioural trials with and without predator odours (2 days), then a chronic water treatment during 8 days (water restriction or water control) followed by a repetition of behavioural trials with and without predator odours (2 days)

### 2.3. Behavioural observations

During each observation trial (see Figure 1), we quantified the behaviour of lizards with a focal sampling survey every 30 min from 08:30 to 17:00. At each focal sampling, we observed if the individual was “active” (i.e. visible to the observer) or “inactive” (i.e., hidden in a shelter or buried in the soil). When the individual was not visible, we searched gently under the shelters and noted if it was found under the wet or dry one. When the lizard was still not found, we assumed that it was buried into the soil and did not disturb it. In this case, the individual was reported as “hidden”. When it was “active”, we further recorded whether the individual was basking (immobile position oriented towards light bulb under the hot spot), exploring (active movement of any kind) or drinking. We reported in which part of the arena the individual was located (either on the hot part or the cold part away from the hot spots and shelters). We also measured from the same distance the surface body temperature on the back of each active lizard using an infrared thermometer (Raynger MX2, Raytek) following standard protocols and previous work showing a strong correlation between surface and core body temperature (Chabaud *et al.*, 2022).

#### 2.4. Plasma osmolality assays

We measured body mass and collected blood samples from the post-orbital sinus using 1-2 micro-capillary tubes (ca. 20-40  $\mu$ l whole blood) before and after the 8 days of manipulation to measure plasma osmolality, which provides a standardized assay of physiological dehydration. Samples were centrifuged at 11,000 rpm for 5 min to separate plasma from red blood cells. Plasma samples (approx. 10-15  $\mu$ l) were immediately frozen at  $-28^{\circ}\text{C}$  until further analyses in the lab. Then, plasma osmolality was determined using a vapor pressure osmometer (model Vapro 5600, ELITechGroup) with the protocol described in Wright *et al.*, 2013 and adjusted to small plasma volumes (Dupoué *et al.*, 2017). Before analyses, plasma was diluted (1:3) in standard saline solution (Osmolarity =  $280 \text{ mOsm.l}^{-1}$ ) to obtain 2 duplicates per sample (CV = 1,5%). Mean value from the 2 duplicates was used in subsequent analyses. Due to their manipulation, a few samples could not be analysed so we only got 55 out of 72 values for osmolality change, randomly distributed among the sex and water treatment.

#### 2.5. Statistical analyses

Statistical analyses were performed using R statistical software (version 3.6.3; R Core Team 2020). First, intra-individual changes in osmolality and body mass during the manipulation were compared with ANCOVA on linear models including effects of treatment, sex and their interaction and the additive effects of initial value (of osmolality or body mass) and of trial group. Second, inter- and intra-individual variation in different behavioural items and in body temperature was analysed with different statistical methods depending on the behavioural item. We first summed the number of times the behaviour was recorded each day relative to the total number of focal sampling in the day, and calculated relative frequency for 5 behavioural items: (1) the proportion of surveys spent active, (2) the proportion of active surveys spent basking, (3) the choice of a wet shelter (proportion of surveys seen in the wet shelter among surveys in a shelter), (4) the proportion of surveys spent hidden, and (5) the proportion of surveys spent in hot zone. These behavioural items were analysed with generalized linear mixed models using the *glmer* function from the 'lme4' package (Bates *et al.*, 2014) with a binomial family and a logit link. We added the experimental arena and the individual as random factors to account for the inter-individual variability and for potential differences among arenas. Next, body temperatures were analysed with linear mixed models using the *lme* function from the 'nlme' package (Pinheiro *et al.*, 2006). In all cases, we tested the tree way interaction between predator scent trial (absence or presence), water treatment (water restricted or water control) and time period (before or after the manipulation), in addition to additive effects of sex, trial group and

test day sequence (day 1 or day 2). Finally, we analysed emergence hour (first time of the day the individual was observed “active”) with a mixed effect Cox model using the *coxme* function of package ‘coxme’ (Therneau, 2012). This model assumes that the emergence time of the lizard can be best described by a proportional hazards model with a single random intercept per group. We fitted the model with additive fixed effects of the predator scent trial, water treatment and time period and their three-way interactions and additive fixed effects of sex, test day sequence and trial group. Random intercept effects were included for arena and individual groups, respectively.

For each analysis, we built a full model and checked its assumptions with graphical analyses of residuals and predictions, for example to test the Gaussian and homoscedastic distribution of residuals. For *glmer* models, we also performed goodness-of-fit tests to confirm the absence of overdispersion. For Cox models, we used a graphical test of proportional hazards provided with the package ‘survminer’ (Kassambara *et al.*, 2017). Data were slightly overdispersed only for one behavioural item (proportion of surveys spend hidden,  $\chi^2 = 407$ ,  $p < 0.0001$ ), so we used an observation-level random effect to correct it as recommended in this situation (Harrison, 2014). Then, starting with the full model, the best model was selected using stepwise removal of non-significant effects based on standard *F* statistics computed with the *Anova* function from package ‘car’ (Fox & Weisberg, 2011). Results are shown as means  $\pm$ SE unless otherwise stated. If the odor trials have an effect on behavioural items, it should appear in the models as a mean effect independently of time period, as it is designed as a one-time effect. On the other hand, the water treatment is designed as a chronic manipulation, and its potential effect should therefore appear as an interactive effect of water treatment and the time period. In particular, we expect no difference between water treatment groups before the manipulation and potential contrasts after the manipulation (see Figure 1). Finally, interactive effects of water restriction and predator scents presence should appear as a three-way interaction between water treatment, time period and predator scent trial.

### 3. Results

#### 3.1. Effect of water deprivation on plasma osmolality and body mass

Water restriction influenced significantly body mass change, with a negative effect on females (water treatment  $\times$  sex:  $F_{1,64} = 9.65$ ,  $p = 0.0028$ ), water restricted females losing on average  $0.12 \pm 0.03$  g whereas control females gained  $0.04 \pm 0.02$  g (estimate =  $-0.16 \pm 0.04$ ,  $p = 0.0009$ ). However, water treatment did not impact males (estimate =  $0.05 \pm 0.05$ ,  $p = 0.32$ ), male body

masses remained constant. We also found an effect of trial group, animals from trial 2 losing more body mass than the two other trials ( $F_{2,64} = 6.73$ ,  $p=0.002$ ). In addition, water restriction increased osmolality by an average of  $17 \text{ mOsm.kg}^{-1}$  in male and female lizards, whereas non-restricted ones decreased their osmolality by an average of  $2 \text{ mOsm.kg}^{-1}$  (water treatment:  $F_{1,55} = 14.63$ ,  $p=0.0003$ ; initial value:  $F_{1,55} = 65.03$ ,  $p < 0.001$ ).

### 3.2. Changes in thermo-hydroregulation behaviours

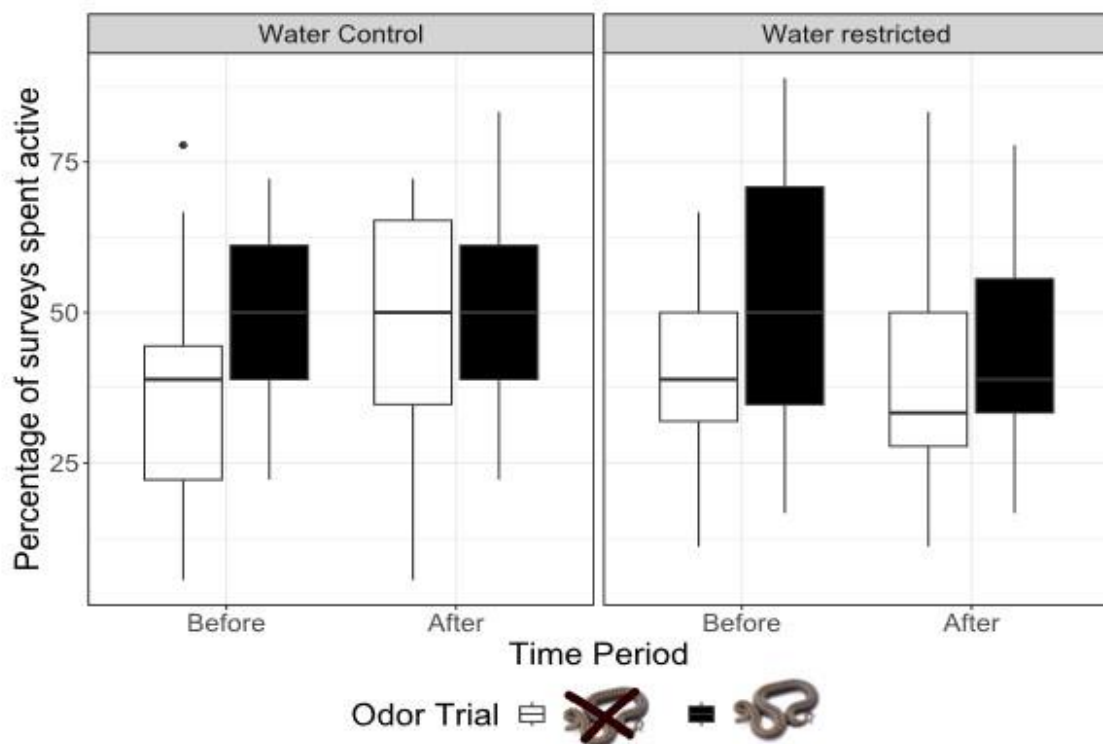
A lizard was reported active on average  $8.4 \pm 0.2$  times a day out of 18 observations. Activity probability increased significantly in the presence of predator scents prior to the water manipulation ( $\chi^2 = 13.23$ ,  $df = 1$ ,  $p = 0.006$ , Figure 2) but this difference was smaller after the water manipulation since lizards were slightly less active in presence of odours after the 8-days manipulation period than before (time period  $\times$  predator scent trial:  $\chi^2 = 3.96$ ,  $df = 1$ ,  $p=0.05$ ). Activity also decreased through time with water restriction compared to the control treatment (time period  $\times$  water treatment:  $\chi^2 = 22.96$ ,  $df = 1$ ,  $p < 0.0001$ , Figure 2) independently from predator scent treatment. Other factors influencing the proportion of time spent active include test day sequence as lizards were less active on the second day of observation trials ( $\chi^2 = 20.95$ ,  $df = 1$ ,  $p < 0.0001$ ).

When lizards were active, the time they spent basking under the hot spot, immobile and exposed was influenced additively by our two experimental constraints as the time spent basking decreased in presence of predator scents (predator scent trial:  $\chi^2 = 5.34$ ,  $df = 1$ ,  $p=0.02$ , estimate =  $-0.37 \pm 0.16$ ,  $p=0.02$ ) and in the water restricted group (time period  $\times$  water treatment:  $\chi^2 = 3.97$ ,  $df = 1$ ,  $p=0.05$ , estimate =  $-0.46 \pm 0.23$ ,  $p=0.046$ ). We also found an effect of time period alone ( $\chi^2 = 10.63$ ,  $df = 1$ ,  $p=0.001$ ) since lizards spent more time basking after than before the manipulation and an overall effect of trial group ( $\chi^2 = 7.96$ ,  $df = 2$ ,  $p=0.019$ ). In addition, when lizards were active, they were situated in the hot part of the arena on average  $11.4 \pm 0.2$  times a day. The predator odour trials influenced the time spent in the hot zone ( $\chi^2 = 15.99$ ,  $df = 1$ ,  $p < 0.0001$ ) but not the water treatment. Test day sequence also impacted the time spent in hot zone ( $\chi^2 = 6.87$ ,  $df = 1$ ,  $p=0.009$ ) as well as the trial group ( $\chi^2 = 7.35$ ,  $df = 2$ ,  $p=0.025$ ). Lizards were less often seen on the hot part of the arena in presence of predator odours and during the second day of the observation sequences.

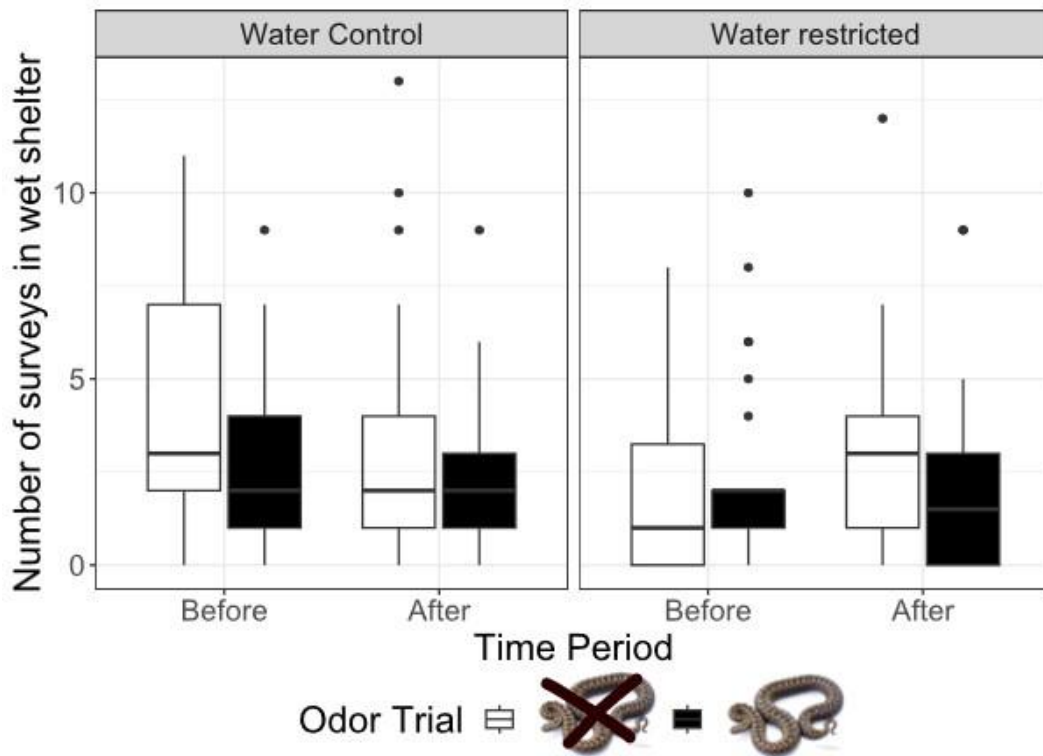
Lizards were found on average  $3.8 \pm 0.2$  times a day under a shelter and only slightly more than half of the time under the wet shelter ( $2.18 \pm 0.15$  times a day). The water treatment influenced the proportion of time lizards spent under the wet shelter differently between the

predator scent trials (time period  $\times$  water treatment:  $\chi^2 = 22.92$ ,  $df = 2$ ,  $p < 0.0001$ ; time period  $\times$  water treatment  $\times$  predator scent trial:  $\chi^2 = 14.11$ ,  $df = 2$ ,  $p = 0.0009$ , Figure 3). In the water restricted group, lizards were seen more often under the wet shelter than in control group when no predator odour was present (estimate =  $1.04 \pm 0.51$ ,  $p = 0.044$ ). Wet shelter preference was not found when shelters and the substrate had predator odours.

Lizards were hidden in the soil on average  $5.8 \pm 0.2$  times a day. Water treatment did not explain the variation in the number of times they were buried (time period  $\times$  water treatment:  $\chi^2 = 2.12$ ,  $df = 2$ ,  $p = 0.34$ ) but the interaction between the predator scent and time period did (predator scent trial:  $\chi^2 = 0.42$ ,  $df = 1$ ,  $p = 0.52$ ; time period  $\times$  predator scent trial:  $\chi^2 = 5.38$ ,  $df = 1$ ,  $p = 0.02$ ), lizards being more often buried in presence of predator odours after the manipulation. The other factor influencing burrowing included test day sequence ( $\chi^2 = 26.70$ ,  $df = 1$ ,  $p < 0.0001$ ), lizards being more often buried on day 2 than on day 1 of behavioural trials. We found no effect of sex on either of the thermo-hydroregulation behaviours.



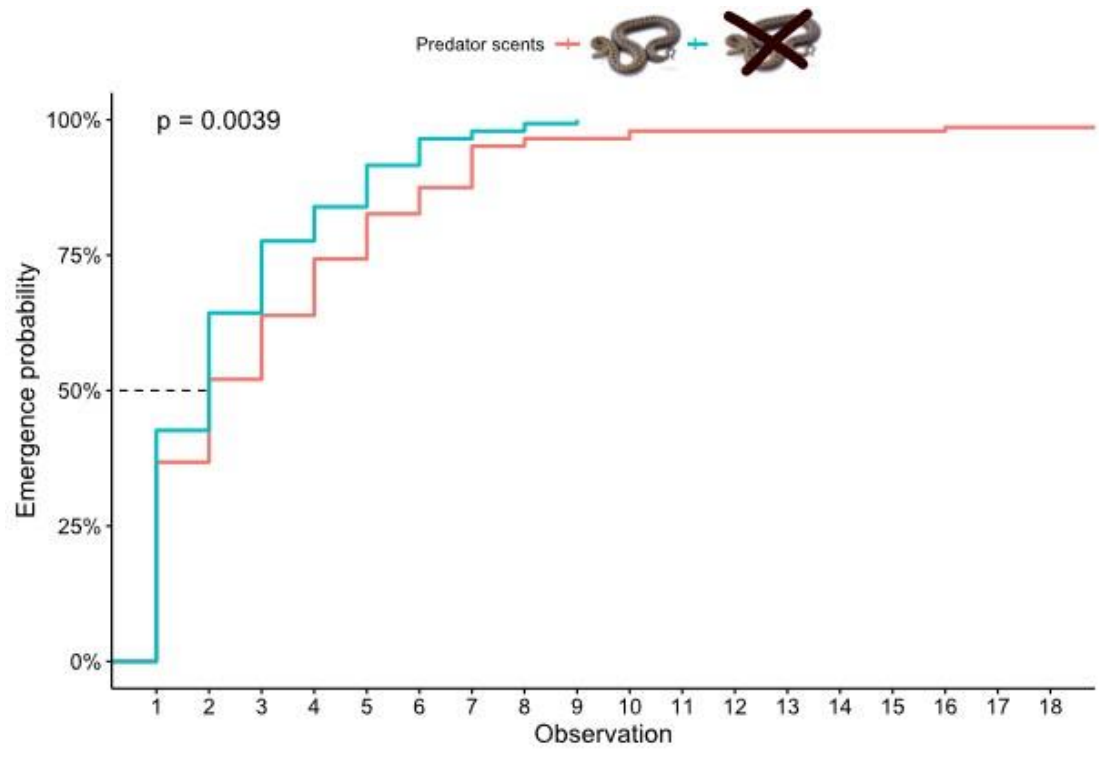
**Fig.2** Proportion of time spent active between 8:30am and 5:00pm in water treatment groups: water control lizards on the left panel and water restricted lizards on the right panel. Behavioural trials are separated according to the presence (black) or absence (white) of predator scents in the environment. Data are reported for measurements “before” the start of the water restriction manipulation, and “after” the end of the water restriction manipulation



**Fig.3** Number of observations in wet shelter between 8:30am and 5:00pm depending of water treatment groups: water control lizards on the left panel and water restricted lizards on the right panel. Behavioural trials are separated according to the presence (black) or absence (white) of predator scents in the environment. Data are reported for measurements “before” the start of the water restriction manipulation, and “after” the end of the water restriction manipulation

### 3.3. Change in emergence time

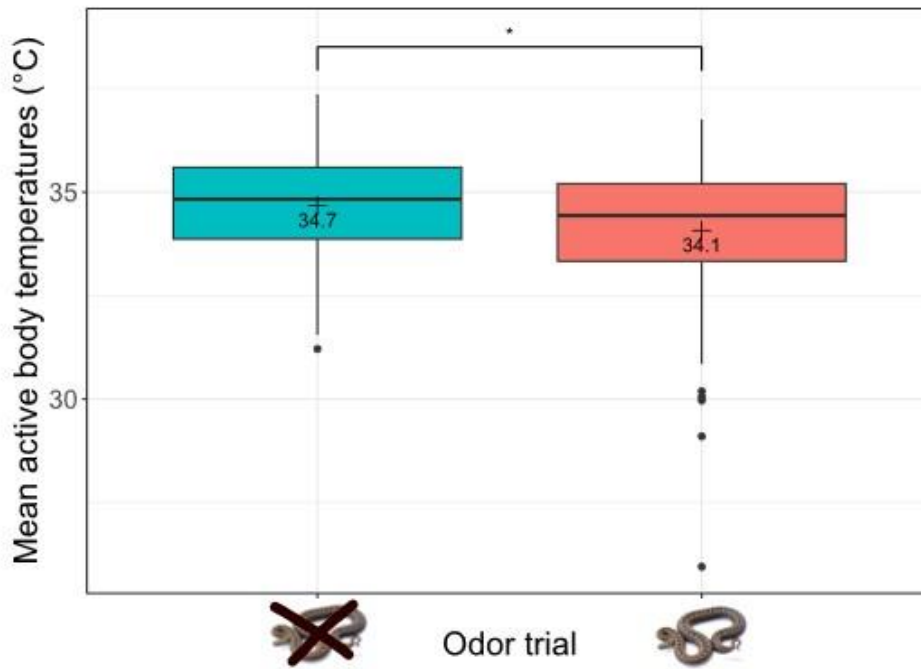
On average, most lizards emerged between the 2<sup>nd</sup> and the 3<sup>rd</sup> observation of the day (9:00 to 9:30 am) with 50% of individuals being active by 9:30 am (Figure 4). Emergence time was influenced significantly by the predator scent presence and its interaction with time period (predator scent trial:  $\chi^2 = 8.79$ ,  $df = 1$ ,  $p=0.003$ ; time period  $\times$  predator scent trial:  $\chi^2 = 9.29$ ,  $df = 1$ ,  $p=0.0023$ ). Lizards emerged slightly later in the presence than in the absence of predator scents (Figure 4), but this was less the case after the manipulation irrespective of the water treatment group. We also found an effect of the test day sequence ( $\chi^2 = 6.29$ ,  $df = 1$ ,  $p=0.01$ ), with lizards emerging slightly earlier on the second consecutive day of observation than on the first. The water treatment had no detectable effect on the emergence time (time period  $\times$  water treatment:  $\chi^2 = 0.77$ ,  $df = 1$ ,  $p=0.38$ ). We found no effect of sex on emergence time.



**Fig.4** Emergence time depending on the presence (red line) or absence (blue line) of predator scents from observation 1 starting at 8:30am to observation 18 ending at 5:00pm. Emergence was scored every 30 mins. Representation are Kaplan-Meier survival curves, produced with *survfit* function

### 3.4. Change in body temperature

Mean surface body temperature during activity was  $34.3 \pm 0.1$  °C. The mean body temperature during the day was slightly reduced by the presence of predator odours ( $\chi^2 = 4.2$ ,  $df = 1$ ,  $p=0.04$ , Figure 5) but only marginally by the water restriction (water treatment  $\times$  time period:  $\chi^2 = 2.8$ ,  $df = 1$ ,  $p=0.09$ ). Time period alone ( $\chi^2 = 7.2$ ,  $df = 1$ ,  $p=0.007$ ) and test day sequence ( $\chi^2 = 9.49$ ,  $df = 1$ ,  $p=0.002$ ) also influenced the body temperature, lizards having a higher mean body temperature after the manipulation, but a lower one on the second consecutive day of observation than on the first. We found no effect of sex on the mean body temperature.



**Fig.5** Boxplot of body temperature during the reported active observations depending on the absence (blue) or presence (pink) of predator scents in the arena. The plus sign represents mean value, written just below, and horizontal line is the median value, N=72 individuals observed two times each in both odor trials

#### 4. Discussion

Our experiment was designed to study the simultaneous effects of water availability and predator threat on thermo-hydroregulation behaviours and activity patterns. We used male and female adult lizards but found no effect of sex on either of the behavioural variable, maybe because the manipulation period was outside reproduction season for this species and different trade-offs between water needs and fear of predation between sexes occur only when reproduction is at stake (Dupoué *et al.*, 2018; Rozen-Rechels *et al.*, 2020). We simulated a mild water deprivation using a chronic stress experiment over several days, whereas predation threat was simulated as a one-time effect using behavioural trials in both presence or absence of predator scents during one day. Thus, our design contrasted the short term responses of well-hydrated and dehydrated animals to a punctual occurrence of predator scents, as might occur in natural populations when predators forage over the territories of several preys and patrol more distances than their preys. Thanks to this experimental design, the behaviours of animals were tested before and after water manipulation, and we could control for inter-individual variation



in mean behaviour, including thermo-hydroregulation temperaments (Cote & Clobert, 2006), to strengthen the statistical power. In addition, a chronic exposure to predator scents without direct physical encounters would have been less relevant as animals can learn that the risk is not associated with danger and behavioural responses will thus fade with time (Parsons *et al.*, 2018). We indeed found that some behavioural traits were influenced by a slight effect of time period, possibly explained by habituation to odours of predators; for example, predator scent effects on wet shelter use and emergence time were weaker after the 8-day water manipulation.

Our study reveals interesting results on how predator threat affects activity and emergence time in this terrestrial ectotherm. The presence of predator scents increased activity rate but in the meantime it decreased basking rate during activity. Increased activity can be associated with a higher level of vigilance and also more frequent escape attempts (via scratching behaviours, see Kawamoto, Le Galliard & Badiane, 2021), but this was not correlated with a better thermoregulation. On the contrary, lizards spent less time basking, which is consistent with the observed reduction of mean active body temperature in presence of predation threat. Overall, this suggests that the presence of predator scents led to less optimal thermoregulation. Previous works on other lizard species also demonstrated a reduction of basking behaviours when a predation risk was perceived, both in laboratory experiment (Downes, 2001; Robert & Thompson, 2007) and in the field (Lister & Aguayo, 1992). The lower basking effort and less accurate thermoregulation in the presence of predators scents also conform with predictions of the cost-benefit model of thermoregulation (Huey & Slatkin, 1976) and other similar studies on ectotherms (Herczeg *et al.*, 2008; Gvoždík, Černická & Damme, 2013). These behavioural responses may represent a significant fitness cost over the long term if maintenance of an optimal body temperature is important for the detection of future threat or foraging efficiency (Amo *et al.*, 2004). For example, a reduction of basking effort had consequences on juvenile growth rates in one study due to the reduced time spent in temperature-dependent activities such as foraging and digestion (Downes, 2001).

We also found that lizards emerged later in presence of predators scents, which might be a strategy to shift their activity toward periods of the day with a more limited predator activity or to reduce overall daytime activity period. This shift of the daily activity pattern is interesting given that smooth snake emerge earlier in the morning during the hottest months of the year (de Bont, van Gelder & Olders, 1986), so emerging later could be a strategy for common lizards to limit encounters with this specialized predator. Prey often need to adjust their behavioural decisions and activity patterns according to geographic variation in predation risks (reviewed

in Lima & Dill, 1990). Sometimes, activity switch from nocturnal to diurnal can even evolve in order to avoid specialized predators (e.g., in a mammalian population Fenn & Macdonald, 1995; in a snake Webb & Whiting, 2005). This shift is particularly important to study for lizards in natural conditions as during the summertime, when drought is combined with high temperatures, activity in the morning is favoured to avoid heat stress and dehydration (Rozen-Rechels *et al.*, 2020).

In addition, our investigation of behavioural changes in water restricted animals mostly confirmed our predictions and previous works on this species (Rozen-Rechels *et al.*, 2020). Dehydration was on average mild in manipulated lizards, with small relative decrease in body mass and small relative increase in plasma osmolality compared to maximum sustainable values that common lizards can tolerate under laboratory conditions (up to 25-30 % body mass decline and up to 50-70 mOsm.kg<sup>-1</sup> increase, see Dupoué *et al.*, 2020). Despite this, dehydrated lizards were significantly less active and had lower basking rates than water control ones, which led to a less accurate thermoregulation given the trend, albeit not significant, for lower body temperatures in the treatment group. Dehydrated lizards also increased their use of the wet shelter, probably as a hydroregulation strategy to reduce evaporative water loss rates and conserve more water (Dezetter, Le Galliard & Lourdais, 2022). Thus, our data confirmed the existence of a behavioural trade-off between thermoregulation and hydroregulation, as predicted for terrestrial ectotherms in Rozen-Rechels *et al.*, 2019 and observed in several recent studies (e.g., Greenberg & Palen, 2021; Nervo *et al.*, 2021). However, these behavioural adjustments did not allow the water restricted lizards to totally compensate for the lack of water, as their plasma osmolality slightly increased compared to non-restricted ones.

From our independent analysis of six behavioural traits and body temperature, we found interactive effects of these two constraints on only one behaviour, which suggests that the two stressors had primarily additive effects on thermoregulation and activity patterns. These conclusions run against our hypotheses that anti-predator responses could depend on hydration state or that thermo-hydroregulation responses are dependent on the fear of predation, as might be the case for example when one environmental constraint has dominant effects on behavioural plasticity and is given priority over the other (Rozen-Rechels *et al.*, 2019). Water stress and the fear of predators additively impacted activity and thermoregulation behaviours, but sometimes in an opposite way. Indeed, as the presence of predator scents in the environment increased activity rate, chronic water stress decreased it; yet, we found no interactive effect. However, water stress and the fear of predators interactively impacted the use of wet shelter, as dehydrated

animals used it more often only in absence of predator scents. In our experimental design, resting under the wet shelter represents a good strategy to optimize both temperature, since the shelter is located in the hot zone closed to the basking area, as well as water balance, since a wet shelter allows lizards to reduce evaporative water losses thanks to the high ambient relative humidity (Pintor, Schwarzkopf & Krockenberger, 2016). However, we used shelters with or without fresh scents of a specialized predator (the smooth snake) implying that shelters were potentially perceived as a danger for dehydrated lizards and priority was given to other, safer behavioural responses to dehydration than shelter use. Thus, these results suggest that a behavioural response to limit dehydration while maintaining a high body temperature (hot shelter use) was modified by the risk of predation (Angilletta, Niewiarowski & Navas, 2002). Similar results on habitat selection were found in geckos, with the avoidance of retreat site with predator scents at the expense of thermoregulation (Downes & Shine, 1998). These results highlight that anti-predator behaviours can have consequences on the regulation of crucial parameters such as body temperature and water balance. If the starvation-predation risk trade-off has been studied in other ectotherm species (e.g., Bennett, Pereira & Murray, 2013), few studies have considered how predation risk can influence the non-energetic costs to the maintenance of water balance (Valeix *et al.*, 2008, 2009a). We need a deeper understanding of the interactive effects of these two constraints in more ectothermic species.

In conclusion, we found mostly additive effects of water and predator threat on thermoregulation behaviours, but an interactive effect was observed for the use of shelter that shift from optimal to sub-optimal in the presence of predators odours. This change in shelter use driven by both water restriction and perception of predations risks could have consequences for thermo-hydroregulation in natural conditions including shelter use during daytime in response to hot temperatures but also shelter use during night time when lizards need to rest at lower body temperatures (Rutschmann *et al.*, 2021). In turn, ecological effects of water restriction should be enhanced in prey species and their predators when they use the same habitat (Valeix *et al.*, 2009a) as it is the case here for common lizards and *Vipera berus*, which live both in the same wet microhabitats (Guillon *et al.*, 2014). Our results suggest that hydroregulation may be critical in lizard populations with a lot of adders as the fear of predation can affect optimal hydroregulation strategies, and “the ecology of fear” (Clinchy *et al.*, 2013) should be taken into account when making predictions about the life history strategies of lizards facing changes in climate conditions.

## 5. Acknowledgements and funding

This study received the financial and technical support from the Centre National de la Recherche Scientifique (CNRS), and was funded by the Agence Nationale de la Recherche under the ‘Aquatherm’ project (ANR-17-CE02-0013 to JFLG). The authors want to thank Théo Bodineau and Jérémy Lefèvre who helped with the experiment and animal care, Elsa Martin for help with the data cleaning and preliminary analyses, as well as the CEREEP Ecotron IleDeFrance staff.

## 6. Compliance with ethical standards

**Conflict of interest** The authors have no competing or financial interests to declare that are relevant to the content of this article.

**Ethical approval** The experimental procedures involving vertebrate animals were carried out in accordance with institutional guidelines and ethical standards, under license from the French Ministry of the Environment (permits APAFIS#25252-2020042722118884). Research protocol was approved by the Darwin ethics committee on animal experimentation, regarding animal welfare.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

## 7. Data availability statement

Data generated during the current study will be deposited in a Zenodo repository after acceptance.

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## **Chapitre 6) Hydric and energetic costs of chemoreception: dehydration alters the response to predator odors in a lacertid lizard**

*In preparation for submission as a note in The American Naturalist*

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**Abstract:**

Understanding the effects of water balance on chemoreception and predator avoidance is crucial for unravelling the ecological adaptations of prey species. Here, we investigated the relationship between chemoreception, water balance, and metabolic rate in a lacertid lizard. Through laboratory experiments and behavioral observations, we measured the hydric and energetic costs of predator detection, and the impact of dehydration on the lizard's response to predator odors as well as its implications for predator avoidance. Our findings revealed that exposure to predator scents led to increased tongue flicking (TF) rates, consistent with previous studies on the same species, which resulted in higher energy expenditure, indicating an energetic cost associated with chemoreception and tongue flicking. Interestingly, although TF rates did not directly affect total evaporative water loss, the presence of predator odors influenced water loss patterns, suggesting behavioral and physiological changes that increased average water loss. Additionally, we examined the effects of individual hydration state on TF rates and its implications for predator discrimination, reaction time, and adequate responses. We found that dehydrated lizards lost their ability to recognize predator scents from control ones, which affected the display of some anti-predator behaviors. Our study highlights the significance of water balance in chemoreception and predator avoidance in lizards. It emphasizes the trade-off between maintaining water balance and effectively responding to predator cues. These findings have broad implications for understanding prey-predator interactions and the sensitivity of terrestrial organisms to water restriction.

Keywords: chemoreception - lizards – dehydration – tongue flick – predation – metabolism

## 1. Introduction

Detecting predators in the environment plays a crucial role in reducing mortality risks. This ability can significantly influence the behavior and distribution of both predators and preys and the dynamics and stability of ecological communities (as reviewed in Lima, 1998). Many prey species have developed chemosensory abilities in addition to sight to detect threats in their environment, enabling them to sample chemicals in their surroundings, which involves different structures in vertebrates and invertebrates (Mathis & Crane, 2017). For example, in squamate reptiles, this additional sensory system relies on the vomeronasal organ or Jacobson's organ (Filoramo & Schwenk, 2009) and olfactory cues are collected via tongue flicks (Schwenk, 1995).

The evolution of chemoreception is facilitated when chemical cues can complement visual cues in dark environments or be used to detect ambush predators that stay invisible. For instance, chemoreception in squamate reptiles has been identified as an ancestral state (Cooper, 1994), and it is associated with the level of sociality (Baeckens & Whiting, 2021), and the foraging mode, with active forager having higher tongue flick rates than ambush ones (Baeckens, Van Damme & Cooper Jr., 2017). Squamate reptiles can use this sense to detect preys, find reproductive partners (Martín & López, 2000), or to avoid predators (Van Damme *et al.*, 1995). The chemical cues released by potential predators will then activate associated anti-predator behaviors like flight (Graves & Halpern, 1990). Previous research has shown that most squamate species are able to differentiate predator scents from control odors or odors from harmless animals (e.g., Cabido *et al.*, 2004; Amo, López & Martín, 2004). Tongue-flick rate (TF rate, i.e. the number of times an animal flicks its tongue to gather chemical cues per unit of time) is classically measured to study the intensity of chemosensory investigation and environmental factors that influence this sensory system (Cooper, 1994). For example, abiotic variables such as temperature can affect TF rate and other anti-predator behaviors, as previous work showed that TF rate increased with higher environmental temperatures close to the thermal preference (Van Damme *et al.*, 1990; Lelièvre *et al.*, 2010).

Although the costs for signalers that emit chemicals have been investigated in details (Johansson & Jones, 2007), the environmental and individual constraints that influence chemoreception abilities to detect olfactory signals inadvertently released by preys, predators or conspecifics are still poorly understood. Yet, models that attempt to predict the optimal design of chemosensory systems assume significant costs of chemoreception (Alberts, 1992). These costs can be temporal as engaging in chemical exploration of the environment can result

in lost opportunities for thermoregulation, mating, or foraging activities, but one untested hypothesis is that chemosensory investigation is also constrained by a range of energetic and non-energetic costs (Mathis & Crane, 2017). In squamate reptiles, tongue flicking is likely to be a relatively high-energy demanding behavior. Some species can flick their tongues several times per second, and energy is required to produce and coordinate the movement of the tongue (Evans, 2003). This rapid movement and associated muscular activity can result in significant energy expenditure, particularly when performed over extended periods of time. In addition, water-based tradeoff could also constrain TF rate because the opening of the mouth, extrusion of the tongue, production of saliva and other secretions required for chemoreception can result in potential hydric costs relative to baseline levels (Loughran & Wolf, 2023). Thus, improved detection and discrimination of chemical signals will increase evaporative water loss, potentially impacting the reptiles' ability to regulate their water balance. If chemoreception induces significant water loss, dehydrated animals should reduce their TF rate, which would impact their ability to detect predator scents in the environment.

To test these hypothesis, we performed two laboratory experiments on the common lizard *Zootoca vivipara*, a widespread species adapted to cold and wet environments and that relies on TF behaviors to detect and discriminate odors from potential predators (Van Damme *et al.*, 1995). First, we used open-flow respirometry techniques to quantify the increase of energy expenditure and evaporative water loss rates in active lizards exposed to predator scent relative to baseline values. Second, we performed a behavioral study to measure the impact of dehydration on chemoreception abilities as measured by TF rate and other behavioral responses typically associated with the detection of specialized predators in this lizard (Thoen, Bauwens & Verheyen, 1986). Depending on the associated costs of tongue flicking, we predict the following results: (1) if costs of tongue flicking are negligible, we expect that predator cues will have no influence in the metabolic and hydric response nor in the behaviors of dehydrated lizards; (2) if tongue flicking only increases energy expenditure, we predict a correlation between TF rates and metabolic rates in presence of predator odors but no change in chemoreception behavior of dehydrated lizards; (3) if tongue flicking has both hydric and energetic costs, we should find a relationship between total evaporative water loss (TEWL) and TF rates, with an effect of predator odors on TEWL. As a consequence, water restricted lizards should reduce their TF rate when confronted to predator scents compared to well-hydrated ones, with an impact on their ability to discriminate odors and display associated anti-predator behaviors.

## 2. Material and methods

### 2.1. Study species

The common lizard *Zootoca vivipara* is a small Euro-Siberian lacertid lizard (adult snout-vent length 50-70mm) living in mesic environments, such as wet meadows and bogs, where it can reach very high population densities (up to ca. 1,000 lizards per ha). The species is consumed by generalist predators such as wide range of carnivores and birds (Strijbosch 1981), as well as by snake species including the common adder (Viperidae, *Vipera berus*, Prestt, 1971) and the smooth snake (Colubridae, *Coronella austriaca*, Brown et al. 2014). The common lizard is also highly sensitive to water deprivation due to its high standard water loss rates (Lorenzon *et al.*, 1999; Massot *et al.*, 2002; Dupoué *et al.*, 2017). Common lizards used in these two experiments were captured in semi-natural populations maintained in outdoor enclosures in CEREEP-Ecotron IleDeFrance in Saint-Pierre-lès-Nemours, France (48°17'N, 2°41'E). They were maintained in a temperature-controlled room (23°C from 8:00 to 19:00, 15°C otherwise), fed with living crickets (*Acheta domestica*) *ad libitum*, provided with drinking water available *ad libitum* and with water sprayed 3 times a day to maintain a wet environment.

### 2.2 Experiment 1: Evaporative water loss and metabolic measurements

We performed respiratory exchange measurements to measure standard metabolic rate ( $V_{CO_2}$  mL.h<sup>-1</sup>,  $V_{O_2}$  mL.h<sup>-1</sup>) and evaporative water loss rates (TEWL mg.h<sup>-1</sup>) on 20 active adult male lizards using an open-flow respirometry system (as described in Dupoué *et al.*, 2015). We placed each lizard in an individual test chamber (RC-3 Respirometry Chamber 700ml, Sable Systems International), placed inside an environmental chamber where temperature was controlled to 28°C, which is close to the preferred body temperature of active lizards (Gvoždík & Castilla, 2001) and similar to the average field body temperature of adult lizards (Rozen-Rechels *et al.*, 2021). We controlled the humidity in the incoming air with a dew point generator (DG-4, Sable Systems, Las Vegas, USA) set up at a target dew point temperature of 14°C. Influx air (80mL.min<sup>-1</sup>) entering in the respirometry chamber previously passes either through an empty jar or one filled with dry skin shed of common adder (*Vipera berus*) to allow measurement in two conditions: (1) with no particular odor and (2) in presence of “predator scents”. Efflux then passed sequentially through a hygrometer (RH 300, Sable Systems, Las Vegas, USA), a column of dessicant (drierite) and to a FoxBox respirometry System (Sable Systems, Las Vegas, USA) with both CO<sub>2</sub> and O<sub>2</sub> analyzers. Based on evidence that tongue-flick frequency decreases after 5-6 minutes of exposure to predator cues (Van Damme *et al.*,

1990), we recorded output signals during 10 minutes in each condition with a specific interface (UI-3) and the associated data analysis software (ExpeData, Sable Systems, Las Vegas, USA). We graphically selected 8-minute periods representative of each condition (“Baseline” without lizard, “Control” with air passing through an empty jar before reaching the lizard and “Predator odors” with air passing through a jar filled with dry adder molts before reaching the lizard). Lizards went through the experiment twice in each condition and we repeated the measurement a third time when feces were detected. Lizards were also video recorded during the whole process so we can count the number of tongue flicks without them seeing the observer. We then analyzed total evaporative water losses (TEWL;  $\text{mg}\cdot\text{h}^{-1}$ ) and CO<sub>2</sub> production ( $\dot{V}\text{CO}_2$  proxy for SMR;  $\text{ml}\cdot\text{h}^{-1}$ ) using equation adapted to our set up from Withers, 1977 and transformed in mass-relative values.

### 2.3. Experiment 2: Behavioral observations under water stress

We performed behavioral observations on 30 yearling female lizards in two hydration state conditions with a video recording set up and then videos were analyzed blindly with the BORIS software (Friard & Gamba, 2016). We presented three types of odors to the lizard with a cotton swap, following the protocol described in Amo *et al.*, 2004. We recorded their tongue flick rates, the latency time before the first reaction, and four anti-predator behaviors (flight, bite, tail vibration, foot shake, see Thoen *et al.* 1986) in response to a control scent (distilled water), to an harmless snake scent (grass snake, *Natrix natrix*) and to a saurophagous-specialist snake scent (smooth snake, *Coronella austriaca*). Snakes were captured nearby the lizard enclosures a few days before the experiment starts. Individuals were separated in three trial groups (N=10 per group) and each odor was presented two times to each individual over two consecutive days. Then, half of the individuals (N=15) underwent an acute water restriction during 5 days, whereas the other half stayed under optimal water conditions. Before and after this water manipulation, we weighted the lizard with a high prevision scale and bled them to measure plasma osmolality (an indicator of the animal hydration state) with a vapor pressure osmometer (model Vapro 5600, ELITechGroup, as described in Dupoué *et al.* 2017). We then repeated the behavioural experiment two times per odor on every lizard to study the effects of water restriction on these anti-predator responses.

### 2.4. Statistical analyses

Statistical analyses were performed using R statistical software (version 4.2.1; R Core Team 2020).

#### 2.4.1. Experiment 1:

We used linear mixed models (lme, package nlme, Pinheiro *et al.*, 2006) to assess (1) the effect of odor treatment on tongue flick rate and (2) the effect of odor conditions on TEWL ( $\text{mg}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ) and  $\dot{V}\text{CO}_2$  ( $\text{ml}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ ). We added TF rate, chamber temperature, the run number (1, 2 or 3), level of activity (high, normal, low) and presence of feces (absence, little, large) as covariates because they are known to influence either TF rate, TEWL or  $\dot{V}\text{CO}_2$  (Van Damme *et al.*, 1990; Dupoué *et al.*, 2015). We added the identity of lizard as a random factor to account for repeated measurements. We selected the best model by removing the non-significant covariates step by step based on standard  $F$  statistics computed with the *Anova* function from package ‘car’ (Fox & Weisberg, 2011). Based on an examination of the residuals of the full model, we also added a variance structure effect with the varIdent function of package *nlme* to allow different variances in our two odor conditions.

#### 2.4.2. Experiment 2:

First, intra-individual changes in osmolality and body mass due to the water manipulation were compared with ANCOVA on linear models including effects of treatment and the additive effects of initial value (of osmolality or body mass) and of trial group. Second, inter- and intra-individual variation in different behaviors were analyzed with generalized linear mixed models (Bates *et al.*, 2009) with a Poisson distribution, to assess the effect of water treatment in interaction with time and the type of odor on the tongue flick rate and occurrence of anti-predator behaviors. We added the experimental arena and the individual as random factors. In all cases, we tested the three way interaction between odor type (water, grass snake or smooth snake), water treatment (water restricted or water control) and time (before or after the manipulation). Then, starting with the full model, the best model was selected using stepwise removal of non-significant effects as previously described.

### 3. Results

#### 3.1. Hydric and energetic costs of predator detection

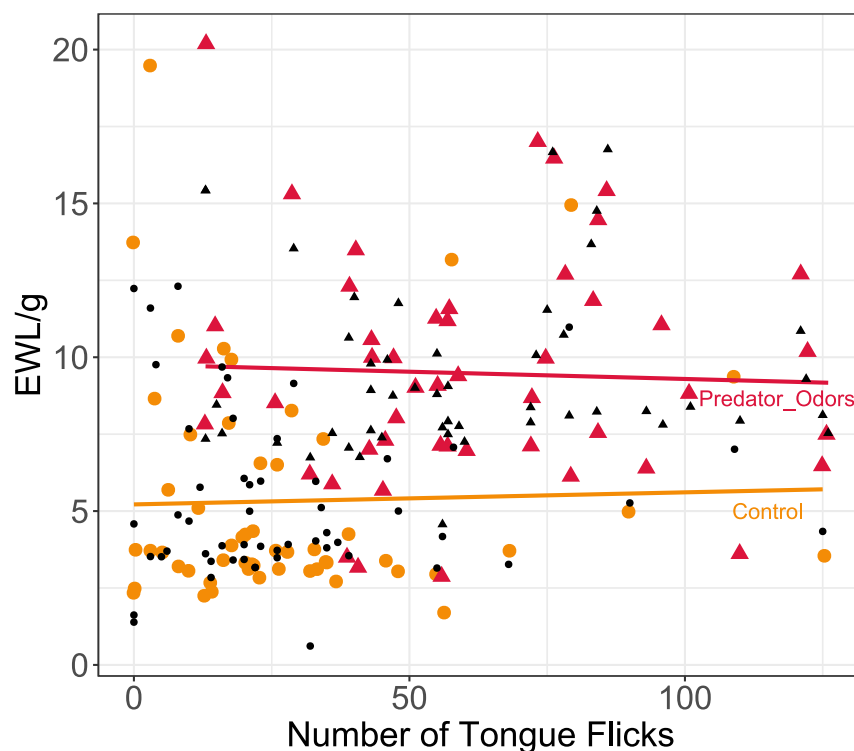
The presence of predator odor increased significantly the TF rate of lizards (odor condition:  $F_{1,79} = 63.8$ ,  $p < 0.0001$ , see Figure 2A & Figure S2) as well as did the level of activity ( $F_{2,79} = 4.4$ ,  $p = 0.015$ ) and also marginally the ambient temperature ( $F_{1,79} = 3.5$ ,  $p = 0.06$ ). The average TF rate nearly doubled between the no odor and the predator scent conditions (contrast =  $31.6 \pm 4.2$  SE,  $p < 0.0001$ ). The variation in TEWL was explained by odor condition ( $F_{1,77} = 76.2$ ,  $p < 0.0001$ , see Figure 1A) but not by the number of tongue flicks ( $p = 0.79$ , see Figure 1A) and



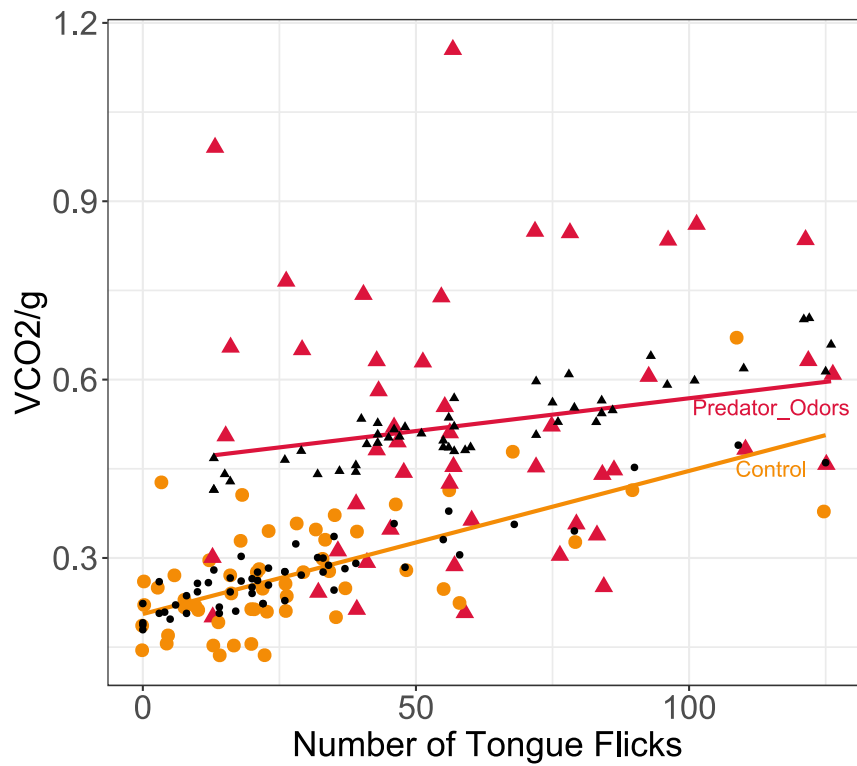
further increased with the level of activity ( $F_{2,77} = 3.6$ ,  $p=0.03$ ) and the presence of feces ( $F_{2,77} = 30.8$ ,  $p<0.0001$ ). The TEWL increased significantly when lizards were in the presence of predator odor (contrast =  $3.7 \pm 0.6 \text{ mg.g}^{-1}.\text{h}^{-1}$ ,  $p<0.0001$ ) and in the presence of feces (large: contrast =  $5.7 \pm 0.8 \text{ mg.g}^{-1}.\text{h}^{-1}$ ,  $p<0.0001$ ; small: contrast =  $3.9 \pm 0.9 \text{ mg.g}^{-1}.\text{h}^{-1}$ ,  $p=0.0001$ ). At the same time, the  $\dot{V}\text{CO}_2$  also increased when lizards were confronted to predator odor ( $F_{1,80} = 64.5$ ,  $p<0.0001$ , contrast =  $0.19 \pm 0.03 \text{ ml.g}^{-1}.\text{h}^{-1}$ , see Figure 1B) and further increased with the TF rate ( $F_{1,80} = 23.4$ ,  $p<0.0001$ , slope =  $0.002 \pm 0.0004 \text{ ml.g}^{-1}.\text{h}^{-1}$ , see Figure 1B). The best model for  $\dot{V}\text{CO}_2$  also allowed for higher residual variance in the presence of predator odor relative to control conditions (LRT = 47.3,  $p<0.0001$ ). TEWL and  $\dot{V}\text{CO}_2$  were positively correlated but the intercept varied according to odor condition (Figure S1).

**Figure 1: Relationship between evaporative water loss (EWL) and tongue flick rate (TF) and between rejected volume of CO<sub>2</sub> (VCO<sub>2</sub>) and TF rate, according to the two different odor treatment (No odor with orange dots and predator odors with red triangles). A.** Plotted data in colored points are raw data and black points are the fitted values of the best model which includes odor treatment, TF, activity level and presence of feces as explanatory variables and a random individual effect. **B.** Plotted data in colored points are raw data and black points are the fitted values of the best model which includes odor treatment and TF as explanatory variables, a random individual effect and an adjustment for different variance according to odor treatments.

A.

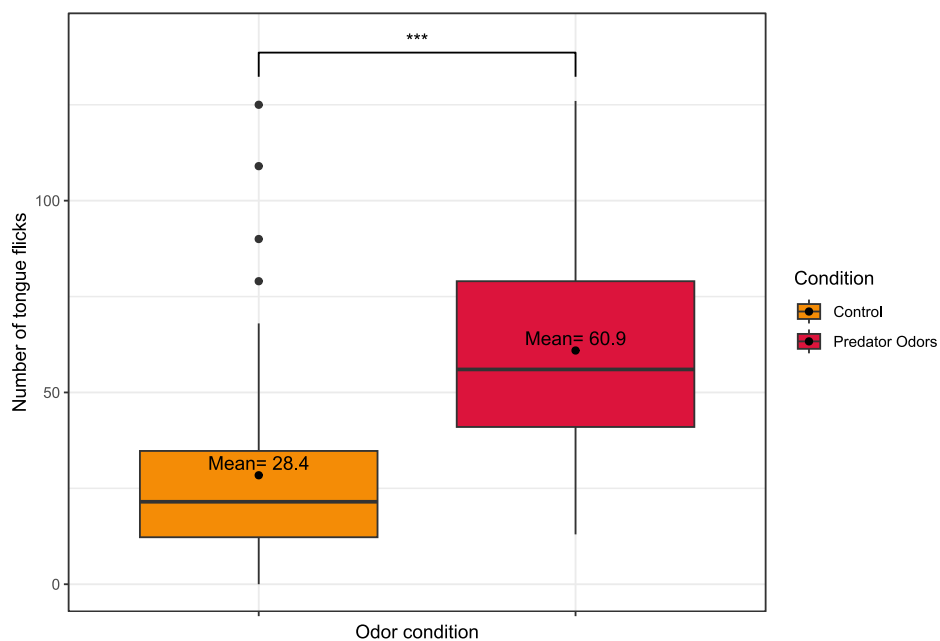


**B.**

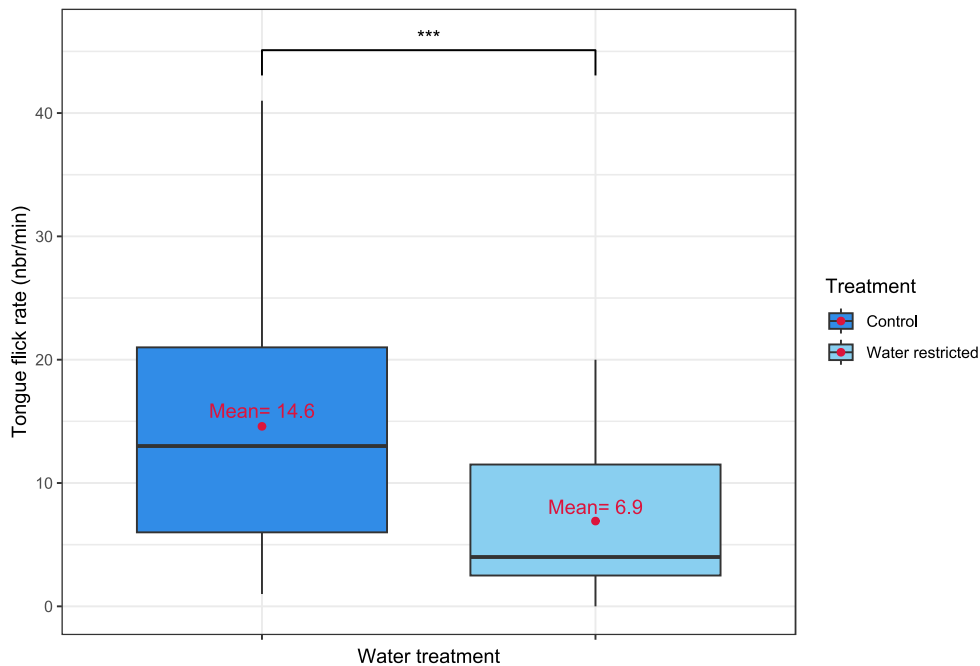


**Figure 2:** Boxplot of mean tongue flick rate: A. In presence or absence of predator scents ; B. In water restricted or water control animals. Mean values are represented by a dot and numerical values written above it.

**A.**



## B.



### 3.2. Water restriction affects predator detection

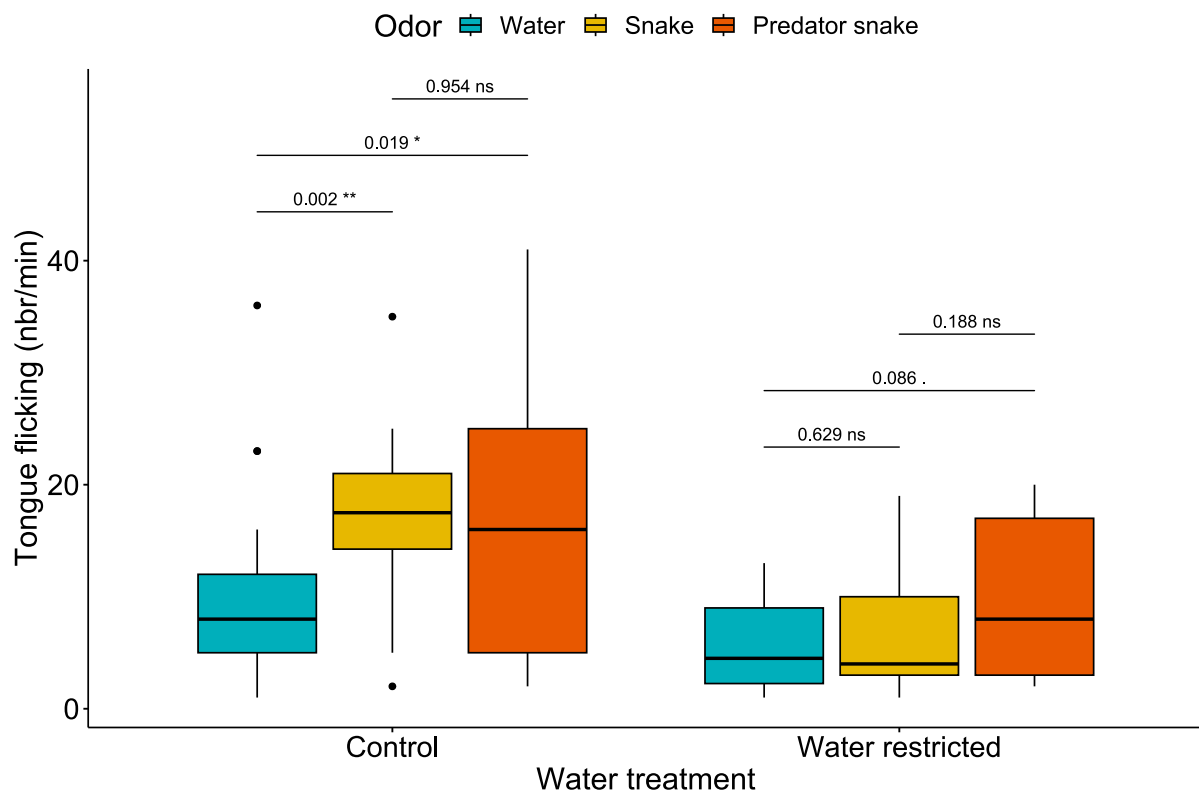
Water restriction significantly reduced body mass (water treatment:  $F_{1,29} = 87.0$ ,  $p < 0.0001$ , initial BM:  $F_{1,29} = 4.0$ ,  $p = 0.056$ , see Figure S3A) and increased importantly plasma osmolality (water treatment:  $F_{1,26} = 113.2$ ,  $p < 0.0001$ , see Figure S3B). Control females maintained a constant body mass and plasma osmolality, whereas, in water-restricted females, plasma osmolality increased on average by  $101 \pm 9$  mOsm/l and body mass decreased by ca. 418 mg (~9% of initial body mass), thus indicating strong physiological dehydration.

The number of TF, independently of odor type, was influenced by the water treatment (water treatment x time:  $\chi^2 = 61.2$ ,  $p < 0.0001$ , see Figure 2B). The mean number of TF for water restricted animals was 10.4 during the 1-minute period of our video when it was 15.0 for well-hydrated ones, whereas the two groups had similar values 6.9 at the beginning of the experiment. In addition, the odor type influenced the TF rate, in interaction of water treatment (water treatment x time x odor:  $\chi^2 = 16.5$ ,  $p = 0.00025$ , see Figure 3). Post-hoc tests showed that the difference between odor types seen with control lizards was almost lost in dehydrated lizards (see Figure 3). In particular, there was no significant difference between control odor and snake odor in water restricted animals ( $p = 0.63$ ) contrary well-hydrated animals ( $p = 0.002$ ).

In addition, the number of behavioral flights response was also impacted by the water treatment (water treatment x time:  $\chi^2 = 5.8$ ,  $p = 0.016$ ), as by the odor type (odor:  $\chi^2 = 12.4$ ,  $p = 0.002$ ) but not by the interaction of both ( $p = 0.9$ ). The lag time between the odor presentation

and the first TF was influenced by the interaction of water treatment and odor type (water treatment x time x odor:  $\chi^2 = 16.6$ ,  $p=0.0002$ ). After water manipulation, this lag time was on average 5.5 seconds for well-hydrated lizards but 13 seconds for dehydrated ones.

**Figure 3:** Boxplot of mean tongue flick rate per odor types in well-hydrated and water restricted lizards. Post hoc tests are represented with the p-value above the different groups.



#### 4. Discussion

Our study provides the first investigation of the relationship between chemoreception, water balance, and metabolic rate in lizards. The presence of an odor of a predatory snake was associated with increased TF rate in the two laboratory experiments as found in previous studies of the same species (Thoen *et al.*, 1986; Van Damme *et al.*, 1995). In addition, we found that the presence of the predator's odor led to a higher energy expenditure and that TF rate was additionally correlated with an increase in activity metabolism in both conditions, indicating a significant energetic cost to chemoreception as well as an energetic costs of tongue flicking. Interestingly, total evaporative water loss (TEWL) was not directly affected by the number of TF but was also influenced by the presence of the predator's odor. Thus, exposure to predator scents led to behavioral changes that increased water loss on average, but we could not directly attribute this non-energetic costs to the increasing TF rates alone.

There are several possibilities to explain these findings. First, the potential impact of other factors, such as physiological stress and increased activity, on water loss in the presence of predator cues (Gangloff & Greenberg, 2023). In particular, increased respiratory frequencies caused by the fear of predation could raise water loss independently of TF (Riddell *et al.*, 2018). Cutaneous water loss could also be affected by the physiological state induced by the presence of predator scents in the immediate surroundings, as it is a plastic trait with intra-individual variations caused by environmental factors (Weaver *et al.*, 2022).

Second, we chose to analyze the average TEWL, VCO<sub>2</sub> and TF rates during the 8-minute period of observation, but we could look more precisely at the TF peaks induced by the detection of scents and the associated increases in air humidity, thanks to the video recordings. This would allow us to obtain a higher precision of the water loss induced by the TF peak. Third, since we observed a difference in the intercept for the relationship between TF and CO<sub>2</sub> release and TF and TEWL, we hypothesized that this may be due to differences in the nature, intensity, and duration of the TF between the two odor conditions (Bryant, Bateman & Fleming, 2011).

In our second behavioral experiment, we successfully induced a dehydration state in water-restricted lizards, as evidenced by a significant increase in their plasma osmolality corresponding to a severe dehydration (Dupoué *et al.*, 2017). In turn, we found that dehydrated lizards had a much lower TF rate than well-hydrated ones, as they reduced the average number of TF per minute by half. This reduction in TF could be seen as a hydroregulation strategy (Pirtle, Tracy & Kearney, 2019). It implies the existence of a tradeoff between the maintenance of water balance and the avoidance of predators, as reduced TF rates impaired the ability of lizards to differentiate predator scents from control odor, delayed their reaction to a predator threat and reduced the intensity of other anti-predator behaviors such as flight.

Overall, our results suggest that water stress can impact the ability of lizards to chemically assess their surrounding environment, which can have significant consequences for predator avoidance and therefore their survival. As chemoreception is widely used by terrestrial organisms for foraging and avoiding predators (Mathis & Crane, 2017), these findings have global implications for sensitivity to water restriction. Further studies are necessary to investigate how the hydric environment mediates prey-predator interactions. For instance, air humidity (water vapor deficit) can play an essential role in chemoreception, as low humidity increases the evaporation rate of chemical cues, reducing their persistence and making them less available for detection, leading to reduced sensitivity to chemical cues (Alberts, 1992) and low humidity challenges the chemoreception abilities of lizards (this study). On the other hand,

high humidity can dilute the concentration of volatile chemical cues, making them less available for detection (Wilder *et al.*, 2005). For reptiles and other animals that rely on chemoreception to detect chemical cues in their environment, air humidity should thus be an essential abiotic factor influencing their capacity for predator avoidance. In addition, predator snakes also rely on chemoreception to detect their preys (Pernetta, Reading & Allen, 2009), which means that the impact of drought could affect both prey and predator species in the same manner (Madsen *et al.*, 2023). While reduced chemoreception ability in lizards could hinder their ability to avoid predators, the reduced capacity for predators to detect their prey could balance this out. Thus, further studies are needed to compare the effect of water restriction on chemoreception ability in different squamate species with varying degrees of sensitivity to water restriction (Le Galliard *et al.*, 2021).

In conclusion, our study highlights the importance of water balance for chemoreception and predator avoidance in lizards. We believe that our findings have broad implications for many prey taxa that rely on chemoreception for predator detection (Kats & Dill, 1998), and further research is necessary to fully understand the impact of water restriction on chemoreception in different species.

## **5. Acknowledgements and funding**

This study received the financial and technical support from the Centre National de la Recherche Scientifique (CNRS), and was funded by the Agence Nationale de la Recherche under the ‘Aquatherm’ project (ANR-17-CE02-0013 to JFLG), the Conseil Régional de Nouvelle-Aquitaine under the ‘Aquastress’ project (2018-1R20214 to O.L.). The experiments were carried out in accordance with institutional guidelines, under license from the French Ministry of the Environment. The authors want to thank Anna Marcout who helped with the second experiment and animal care, and Lauryn Trouillot for her help with the video and preliminary behavioural analyses for the second experiment, as well as the CEREEP Ecotron IleDeFrance and CEBC staff. The authors also thank Anthony Herrel (UMR 7179 CNRS-MNHN) for its loan of respirometry chambers and Mathias Dezetter for the use of viper molts collected during his research work.

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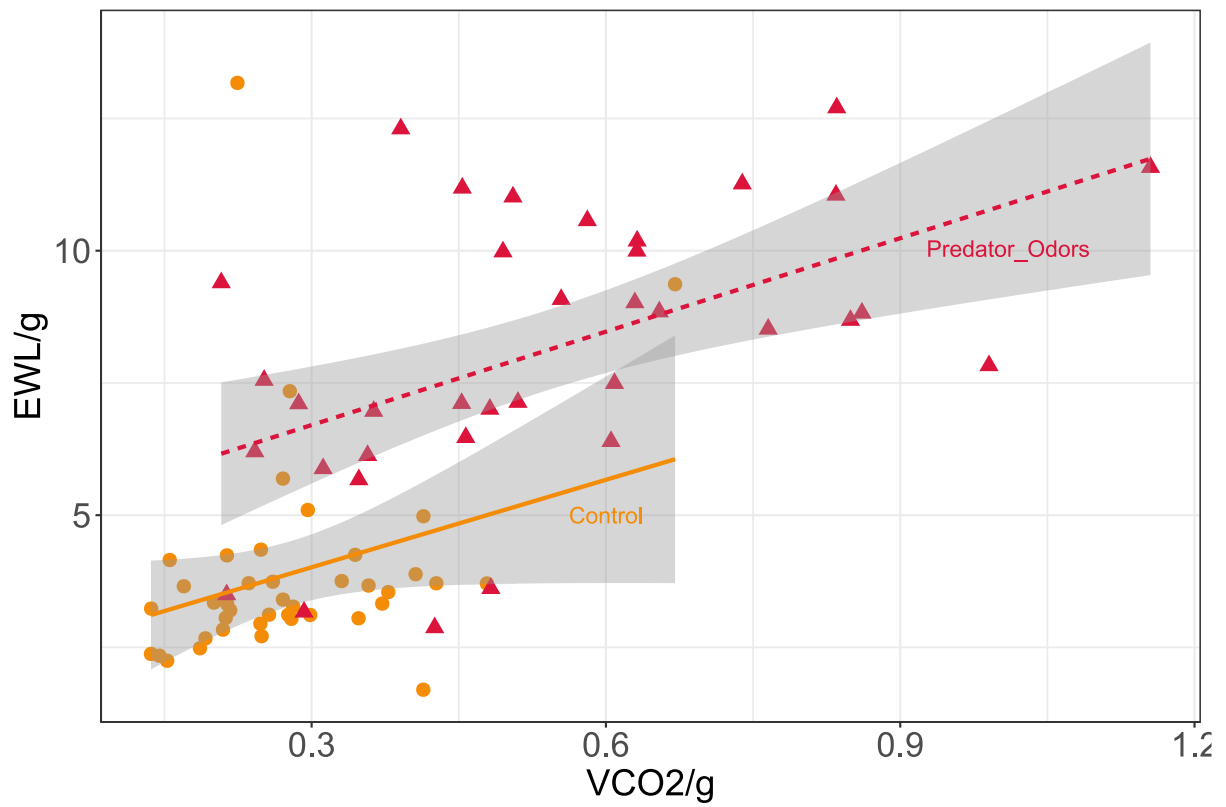
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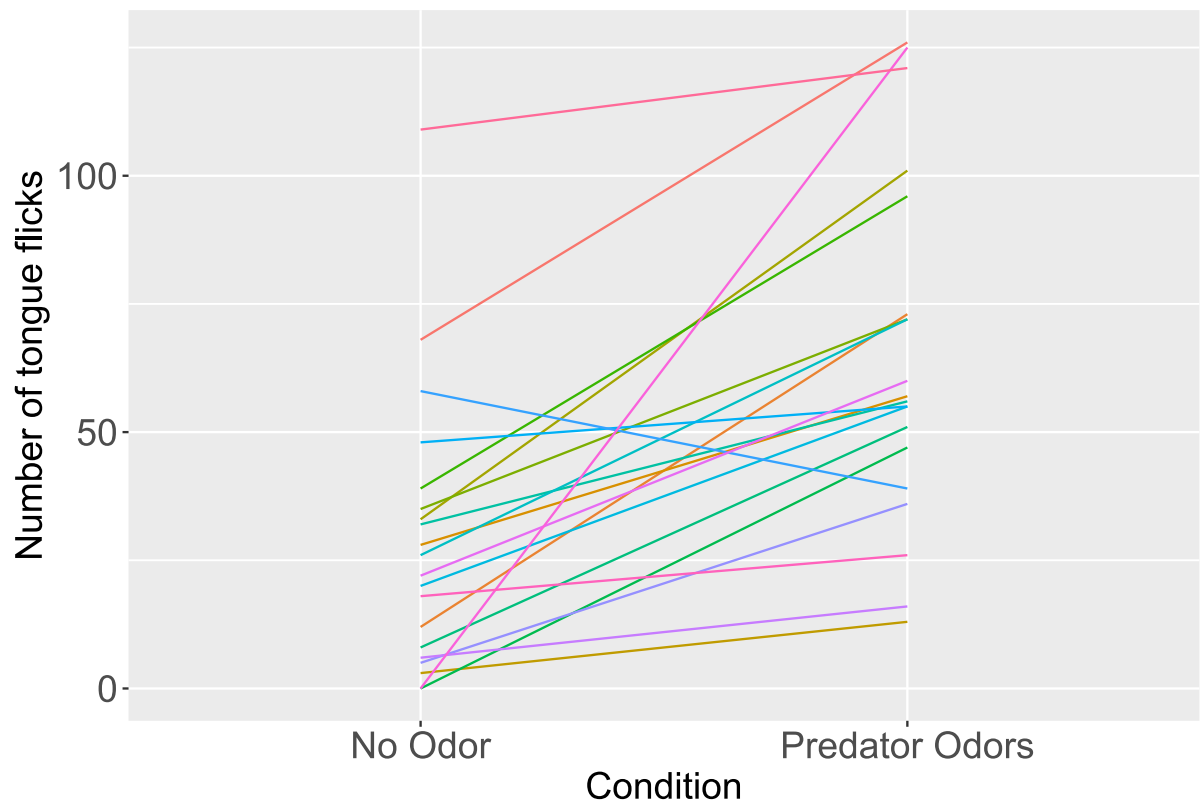
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## Supplementary figures



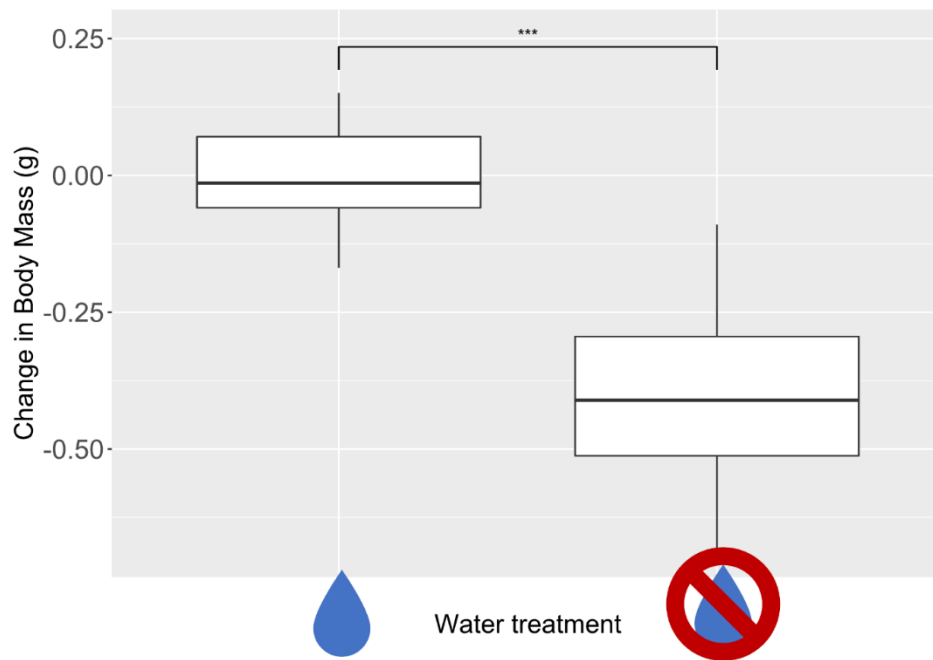
**Figure S1:** Relationship between EWL corrected by body mass and VCO<sub>2</sub> corrected by body mass in two different odor conditions (orange dots with control odor and red triangles with predator odors).



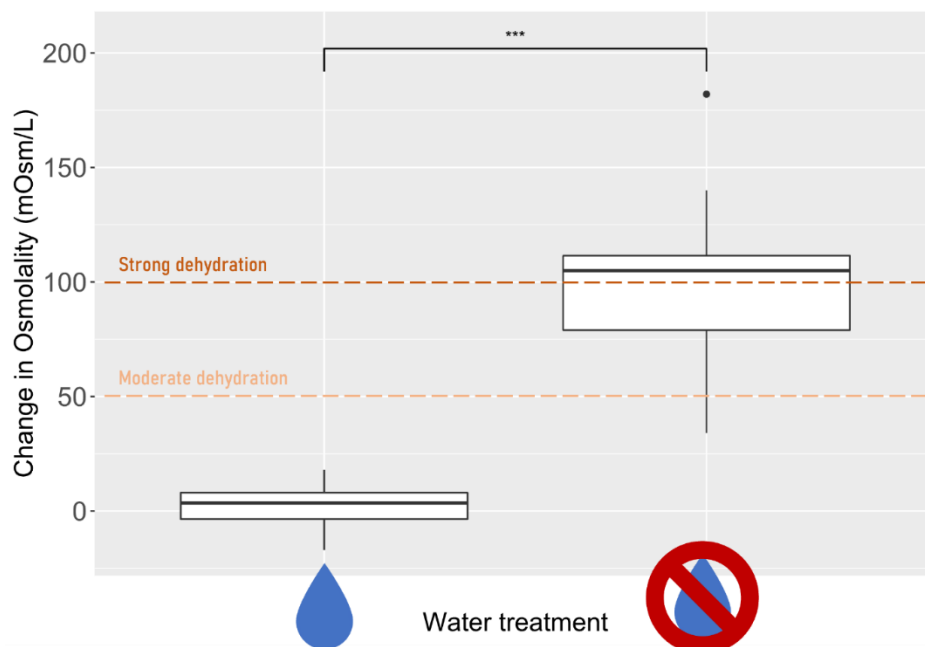
**Figure S2:** Evolution in the number of tongue flicks for lizards in absence (left) or presence (right) of predator odors. The increase in TF was not correlated to the basal TF rate.

**Figure S3:** Change in lizards' body mass (A) and plasma osmolality (B) in two water treatments: water control animals (N=15) on the left and water restricted animals (N=15) on the right.

**A**



**B**





## **DISCUSSION ET PERSPECTIVES**



## 1) Variation des traits de thermo-hydrorégulation

La première partie de ce travail (Partie 1) m'a permis d'améliorer les connaissances sur la diversité des traits liés à la balance hydrique chez les reptiles squamates et leurs variations inter et intraspécifique. Dans le **Chapitre 1**, nous avons souligné le manque de données accessibles et facilement utilisables sur les traits fonctionnels liés à la régulation de la balance hydrique, et nous proposons une base de données globales pour remédier à ce problème. Nos données de pertes hydriques évaporatives rassemblent des études publiées et des données personnelles sur plus de 300 espèces réparties dans plus de 35 pays d'Asie, Afrique, Europe et Amériques, associées à des covariables expérimentales qui peuvent induire des biais dans les analyses qui empêchent souvent la comparaison de données provenant d'études différentes. Cette riche base de données souligne à quel point les reptiles squamates sont des animaux avec une écologie et des aires de distribution extrêmement variées, donc particulièrement pertinents pour des analyses comparatives sur des traits reliés aux conditions climatiques. Ces données ont été mises à disposition pour permettre des analyses comparatives de traits physiologiques en les associant avec le climat dans lequel vivent ces espèces, mais aussi avec d'autres traits tels que le mode locomoteur, le régime alimentaire ou encore l'activité diurne ou nocturne, pour ainsi améliorer les connaissances sur les liens entre balance hydrique et traits écologiques. Il a également été possible de rassembler des données sur le métabolisme car ce sont des aspects intimement liés, les pertes hydriques évaporatives totales (TEWL) incluant les pertes cutanées et respiratoires et parce que des mesures conjointes du métabolisme facilitent le calcul de la résistance cutanée aux pertes hydriques, une mesure standardisée et indépendante du contexte des pertes hydriques (Pirtle, Tracy & Kearney, 2019). Lorsque les données étaient disponibles, nous avons pu renseigner ces différentes composantes des TEWL pour permettre dans le futur d'étudier le rôle de ces différents mécanismes dans la régulation de la balance hydrique, à la fois entre espèces et au sein d'une espèce en utilisant les individus échantillonnés dans des milieux contrastés de l'aire de distribution d'une même espèce. Ces futures analyses permettront de mieux comprendre les adaptations et réponses de ces ectothermes aux changements globaux, car la balance hydrique joue un rôle fondamental dans les performances et la détermination de la niche écologique des reptiles squamates terrestres (Kearney *et al.*, 2018; Gouveia *et al.*, 2019). Par exemple une thèse en cours dans mon équipe de iEES Paris a pour objectif de modéliser l'évolution des pertes hydriques en fonction de l'aridité de l'aire de distribution des espèces. Notre étude soulève tout de même les biais taxonomiques et géographiques des données disponibles, et nous invitons les recherches futures à étudier ces



espèces et régions qui ont été sous échantillonnées par les études passées (notamment Asie et Afrique tropicales).

Dans le **Chapitre 2**, j'ai mené une étude comparative à l'échelle interspécifique (entre le lézard des murailles et le lézard vivipare) et intraspécifique en mesurant les traits morphologiques, physiologiques et comportementaux liés à la thermo-hydrorégulation. L'objectif était notamment de comprendre quelles variables environnementales expliquaient le mieux les variations géographiques de ces traits. J'ai pu trouver des différences entre espèces sur les pertes hydriques totales et cutanées notamment, montrant que le lézard des murailles est plus adapté aux milieux secs et chauds. En revanche, peu d'adaptations physiologiques ou acclimations entre les populations de *Zootoca vivipara*, ont été observées bien que certaines populations aient été échantillonnées en marge sud de la distribution de l'espèce et soient en fort déclin ces dernières années (Cistude Nature, pers. com.) et que nous ayons analysé à la fois des populations à mode de reproduction ovipare et quelques populations vivipares qui sont très éloignées sur l'arbre phylogénétique de cette espèce (Surget-Groba *et al.*, 2001). Il est possible que certaines différences physiologiques ne se manifestent qu'à des périodes précises de la vie, au stade juvénile ou lors de la gestation, et soient donc non visibles dans notre échantillonnage de mâles adultes. Ceci souligne l'intérêt d'avoir des détails expérimentaux (sexe, âge) sur les individus étudiés lors des analyses comparatives entre espèces. Parmi les différences entre populations, notre étude fait cependant ressortir l'importance de la disponibilité en eau dans l'habitat, qui influence davantage les pertes hydriques totales que l'aridité climatique globale ou les températures moyennes. Enfin, le comportement de thermorégulation permet de maintenir une température préférée similaire dans toutes les populations de ce gradient climatique très contrasté. Ce résultat souligne l'importance probable de la qualité de l'habitat à travers notamment des effets Bogert (Munoz, 2022) en permettant de compenser les conditions climatiques extrêmes, comme cela a été montré pour le choix de micro habitats par exemple (Dezetter, Le Galliard & Lourdais, 2022). Prendre en compte les spécificités de l'habitat (comme l'accès à l'eau et le couvert végétal, mais aussi l'hétérogénéité des abris et de la végétation) est donc fondamental pour comprendre les variations de traits écophysologiques.

## 2) Lien entre balance hydrique, alimentation et performances

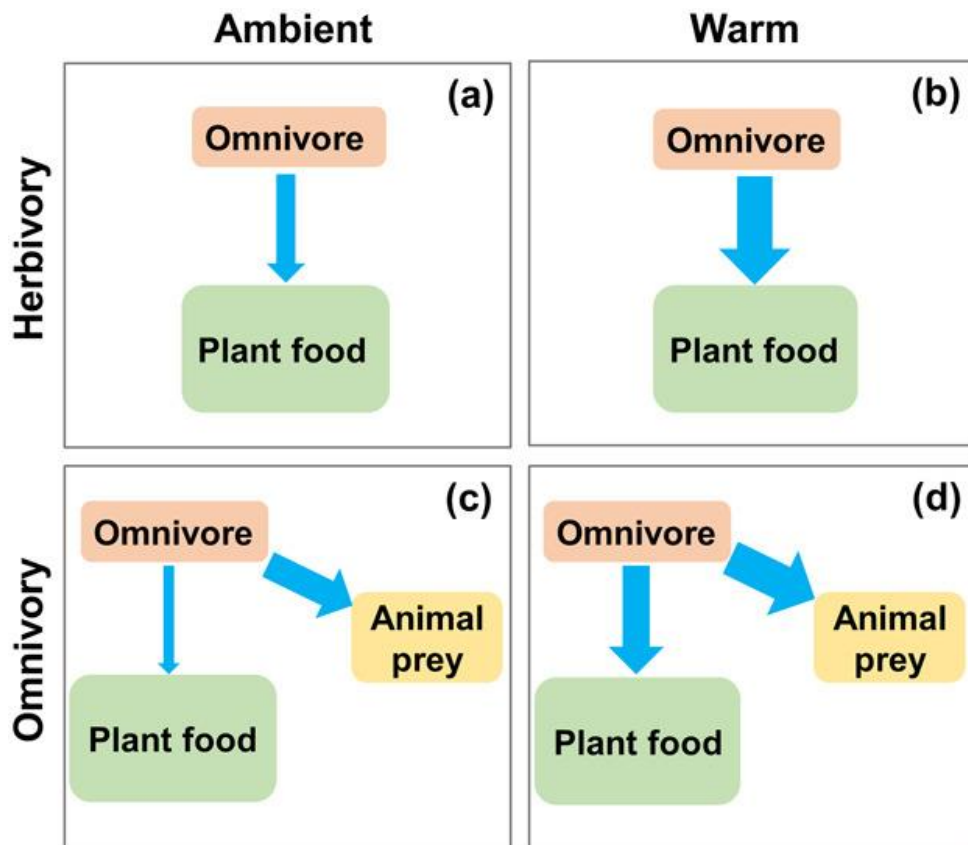
Dans la Partie 2, j'ai étudié comment les apports alimentaires étaient liés à la balance hydrique individuelle, en m'intéressant d'un côté à la composante comportementale avec le **Chapitre 3** et d'un autre côté à des mécanismes physiologiques qui pouvaient l'expliquer dans le **Chapitre 4**. Dans l'étude comportementale du **Chapitre 3**, nous avons cherché à comprendre comment

la qualité hydrique des micro habitats et les ressources alimentaires pouvaient jouer sur les compromis comportementaux de thermo-hydrorégulation mis en place en réponse à une sécheresse. Nous avons montré que les lézards développent des stratégies comportementales pour réduire les pertes hydriques et économiser les ressources énergétiques, comme une réduction de l'activité de basking (se chauffer en s'exposant à la source de chaleur) ou une augmentation de la fermeture des yeux. Mais, si la disponibilité d'un abri humide réduit ces conflits entre hydro et thermorégulation en permettant de maintenir une meilleure condition corporelle et une déshydratation physiologique réduite par rapport à un abri sec, ce n'est pas le cas de l'accès à la ressource alimentaire. Au contraire, devoir assimiler plus de ressources semble augmenter ces conflits comportementaux, car les lézards avec de la nourriture ont passé plus de temps dans le refuge que ceux à jeun, même lorsque celui-ci était maintenu sec. Aussi, les résultats de cette étude ne semblent pas soutenir l'hypothèse selon laquelle la consommation de nourriture représente un gain significatif en eau : l'acquisition de nourriture n'a pas permis de réduire l'augmentation d'osmolalité plasmatique indiquant une déshydratation, et la priorité donnée aux activités liées à l'alimentation a provoqué une plus grande dépendance aux conditions microclimatiques de l'abri, suggérant que le traitement des aliments requiert un bon état d'hydratation.

Afin de comprendre plus intimement ces liens entre prise alimentaire et maintenance de l'état d'hydratation, j'ai mis en place dans le **Chapitre 4** un protocole expérimental original et contrôlé pour étudier si la nourriture pouvait permettre la restauration de la balance hydrique et diminuer les coûts associés à une restriction d'eau. Les résultats de cette manipulation ont montré que manger ne permet pas aux lézards de rétablir la balance hydrique, quelle que soit la qualité hydrique des proies, ce qui peut sembler surprenant et contradictoire par rapport aux prédictions basées sur la littérature. Au contraire, des prises alimentaires plus importantes ont augmenté l'état de déshydratation des lézards, impliquant que la consommation de nourriture compromet l'équilibre hydrique. Ces résultats impliquent que les lézards vivipares dépendent donc d'un accès à de l'eau de boisson pour maintenir un état hydrique optimal. En revanche, l'accès à la nourriture diminue les coûts énergétiques associés à la privation d'eau, tels que la mobilisation de réserves lipidiques ou la déperdition musculaire. Ces résultats suggèrent que les lézards ont privilégié leur balance énergétique plutôt que leur bilan hydrique, bien que des différences de comportements imposées par la digestion (maintenance d'une température corporelle élevée) ont pu accentuer l'effet de la restriction hydrique chez les lézards ayant accès à de la nourriture. Si des études précédentes avaient montré les contraintes hydriques liées à la

prise de nourriture chez les reptiles carnivores (Wright, Jackson & DeNardo, 2013; Lillywhite, 2017), nos travaux montrent que les reptiles insectivores ont également des contraintes physiologiques liées à leur alimentation, notamment des compromis avec le bilan hydrique, ce qui doit être mieux pris en compte dans les modèles cherchant à inclure le budget hydrique dans la prédiction de la distribution des espèces sous le changement climatique (Urban *et al.*, 2016; Kearney *et al.*, 2018; Grimm-Seyfarth, Mihoub & Henle, 2019; Chiacchio *et al.*, 2020). Ces résultats font échos à ceux du **Chapitre 2**, où les lézards des milieux humides étaient en meilleure condition corporelle que ceux des milieux plus arides. Il est possible que ce soit dû à des compromis plus faibles entre thermo et hydrorégulation, et donc à une prise de nourriture plus importante dans ces milieux où l'eau n'est pas une contrainte.

Chez d'autres espèces ectothermes, des changements de régime alimentaire ont été observés, avec des omnivores augmentant leur degré d'herbivorie en réponse à une augmentation des températures (Carreira *et al.*, 2016, 2017; Zhang *et al.*, 2020). Ces études concernent principalement des ectothermes aquatiques, mais l'effet de variables environnementales sur le régime alimentaire des ectothermes terrestres est encore méconnu. Cela pourrait avoir des implications sur les écosystèmes, comme une augmentation de la régulation top-down des niveaux intermédiaires sur les producteurs primaires avec les changements globaux (Zhang *et al.*, 2020, voir Figure 1). La base de données présentée dans le **Chapitre 1** pourrait permettre de tester les liens entre balance hydrique, régime alimentaire et climat afin d'étudier si la proportion de carnivores et insectivores est plus faible dans les milieux où la contrainte en eau est importante. On pourrait aussi tester si les régimes alimentaires carnivores ont par ailleurs des adaptations pour limiter les pertes hydriques par rapport aux herbivores vivant dans des milieux d'aridité similaire, pour compenser ce coût de la prise alimentaire.



**Figure 1** : Possibles effets de l’augmentation des températures sur la consommation de végétaux (producteurs primaires) par les ectothermes omnivores d’après Zhang *et al.* (2020). Dans les scénarios chauds (b et d) ou en absence de proies animales (a), les omnivores augmentent leur niveau d’herbivorie et donc leur effet top-down direct sur les producteurs primaires (largeur des flèches). On pourrait envisager un effet similaire sur les écosystèmes terrestres en remplaçant l’augmentation des températures par la diminution de la disponibilité en eau et/ou de l’humidité ambiante dans la colonne de droite.

### 3) Influence de la balance hydrique sur les comportements anti-prédateurs

Dans la Partie 3, je me suis intéressée à l’autre aspect des relations trophiques du lézard vivipare, soit son comportement face aux prédateurs et comment la balance hydrique intervient dans ces compromis comportementaux. Dans le **Chapitre 5**, j’ai montré que la peur des prédateurs affecte les comportements de thermorégulation du lézard vivipare, de façon majoritairement additive par rapport à la contrainte hydrique mais pouvant aller dans un sens opposé, par exemple sur l’activité. De plus, j’ai trouvé un effet interactif de ces deux contraintes sur le recours à un abri humide, son utilisation n’étant augmentée pour les lézards restreints en eau qu’en absence de prédateur. Cette réponse comportementale qui permet de limiter la déshydratation tout en maintenant une température corporelle élevée a donc été modifiée par le risque de prédation. Cela montre que les comportements anti-prédateurs peuvent avoir des

conséquences subtiles sur la régulation de paramètres cruciaux tels que la température corporelle et l'équilibre hydrique et qu'il est nécessaire de comprendre plus largement comment les proies intègrent différents facteurs de stress biotiques et abiotiques (Grimm-Seyfarth *et al.*, 2019).

Dans le **Chapitre 6**, j'ai étudié la relation entre chémoréception, balance hydrique et taux métaboliques chez le lézard vivipare grâce à deux expériences complémentaires : une utilisant la respirométrie pour mesurer les échanges de gaz et d'eau entre le lézard et son environnement, et l'autre permettant de comparer le nombre de *tongue flicks* (TF) chez des animaux hydratés et déshydratés. Les résultats montrent que l'augmentation de TF en réponse à une odeur de prédateurs augmente le volume de CO<sub>2</sub> rejeté par le lézard, révélant un coût énergétique à la chémoréception. Par ailleurs, les pertes évaporatoires en eau ont aussi été augmentées en présence d'odeur de prédateur, mais elles ne sont pas directement corrélées au nombre de TF. Cela implique que le coût hydrique de la réaction face aux odeurs de prédateurs ne vient pas uniquement de l'intensité de chémoréception mais aussi d'autres facettes du changement physiologique induit par le stress. Les résultats de l'étude comportementale viennent confirmer cela en montrant que des lézards déshydratés diminuent significativement leur nombre de TF. Cela peut avoir des conséquences majeures sur leur survie puisque cette diminution affecte leur capacité à distinguer une odeur de prédateur d'une odeur contrôle ainsi que la mise en place de réponses adéquates.

Pris dans leur ensemble, ces résultats montrent l'importance de la qualité hydrique de l'habitat, à la fois pour expliquer les variations inter-populations de traits écophysiologiques liés à l'hydrorégulation dans le **Chapitre 2**, mais aussi à l'échelle individuelle pour hydroréguler comportementalement (**Chapitre 3**). Ce comportement peut être modifié par les interactions trophiques, comme la prise de nourriture (**Chapitre 3**) ou la présence de prédateurs (**Chapitre 5**). La capacité des lézards à exprimer ces ajustements comportementaux en réponse à un manque de disponibilité en eau dans leur milieu dépend donc à la fois de la disponibilité de ces microclimats dans les habitats mais aussi de leur environnement trophique. Ces travaux ont aussi permis de mettre en évidence que les réponses aux prédateurs, et en particulier la chémoréception, dépendent de l'état hydrique de l'animal (**Chapitres 5 & 6**). Nous proposons une explication physiologique à nos observations comportementales liée aux coûts énergétiques et hydriques de ces réponses, qui n'avaient jamais été évalués jusque-là. Enfin, ces résultats révèlent qu'il peut exister un compromis entre le maintien de la balance hydrique et de la balance énergétique chez les reptiles insectivores des milieux tempérés (**Chapitres 3 & 4**), qui

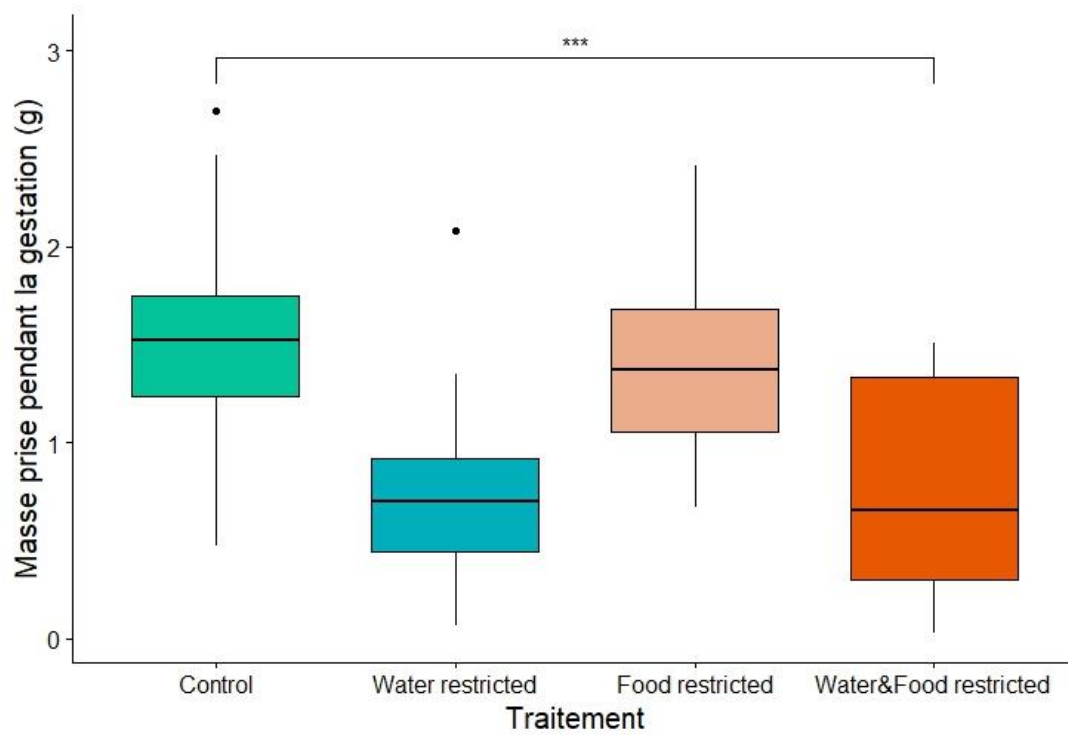
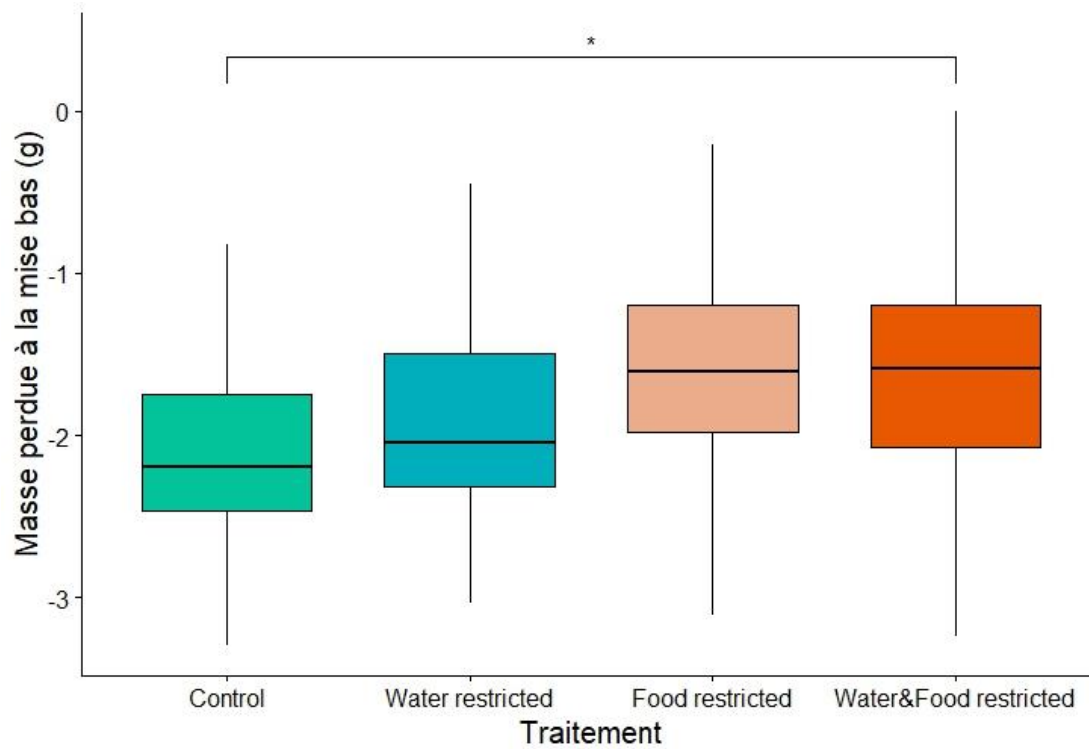
peut être lié au compromis entre thermo et hydrorégulation par les comportements que nécessitent l'assimilation de ressources alimentaires. Ces compromis peuvent se manifester tout particulièrement lors de la reproduction, ce qui nous a amené à réaliser une expérience sur des femelles vivipares gestantes pour étudier les effets conjoints des contraintes trophiques et hydriques lors de la reproduction.

#### **4) Effets conjoints des contraintes trophiques et hydriques sur les performances reproductrices du lézard vivipare**

La reproduction est une étape essentielle du cycle de vie des organismes car elle a un impact direct sur leur valeur sélective et nécessite des apports énergétiques importants. De plus, le développement embryonnaire et les premiers stades de la vie juvénile sont des périodes critiques, qui sont fortement influencées par les conditions environnementales, que ce soit chez les espèces vivipares (Wang *et al.*, 2017; Dezetter *et al.*, 2021; Bruschi *et al.*, 2022), ou chez les espèces ovipares (e.g., Ensminger *et al.*, 2018). Chez le lézard vivipare, les femelles gestantes sont particulièrement sensibles à la restriction hydrique du fait de leur mode de reproduction (Dupoué *et al.*, 2018). Des effets sur la descendance ont aussi été montrés précédemment, la restriction hydrique des mères pouvant influencer le sexe ratio (Dupoué *et al.*, 2019) et générer des comportements anxieux chez les jeunes (Rozen-Rechels *et al.*, 2018). Des études sur d'autres espèces de lézards vivipares ont aussi montré que la restriction alimentaire pendant la gestation avait des impacts sur la qualité des jeunes et leur survie (Wang *et al.*, 2017). On explique cela par le compromis d'allocation des ressources entre la maintenance et la reproduction, des facteurs environnementaux pouvant influencer ces conflits, notamment la disponibilité en eau (Coe & Rotenberry, 2003).

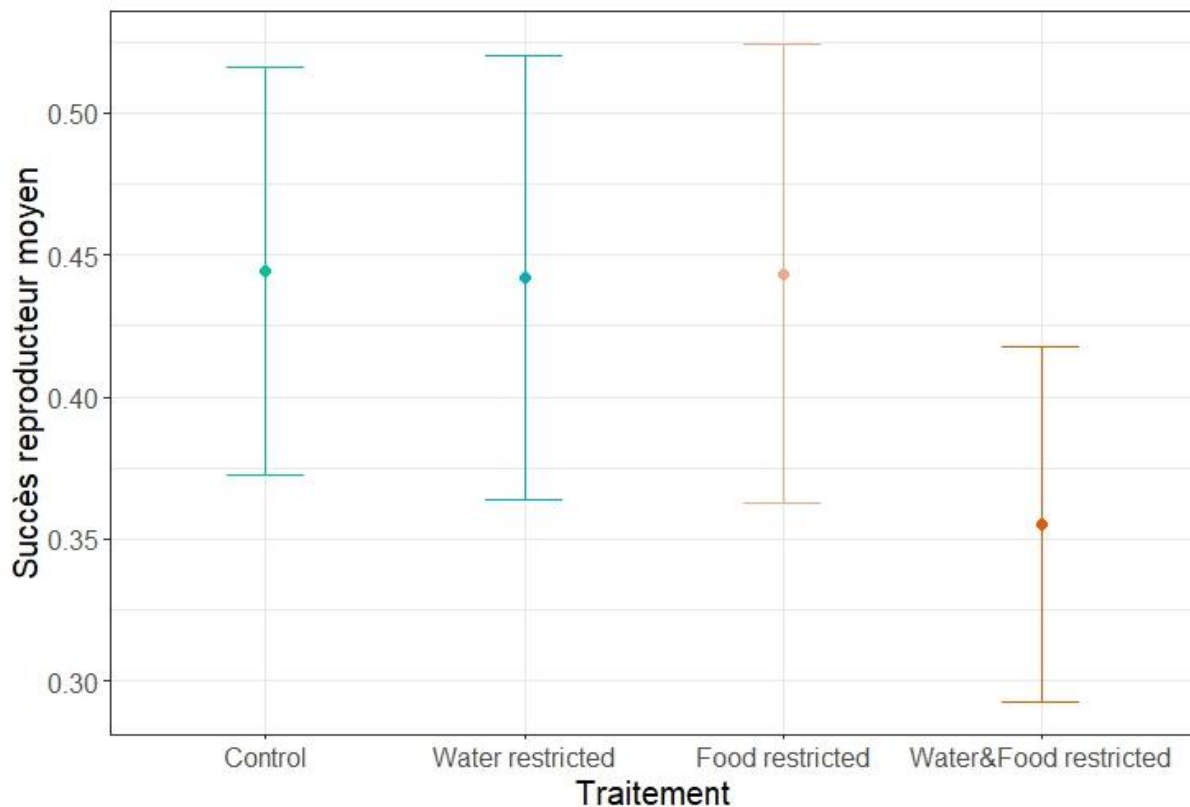
Les changements climatiques en cours et notamment les changements de disponibilité en eau ayant un fort impact sur la survie et la reproduction des insectes (Harvey *et al.*, 2022), les proies d'un mésoprédateur insectivore comme un lézard peuvent venir à manquer en période de sécheresse, et avoir un impact fort sur la reproduction de cette espèce. Aussi, nous avons voulu tester l'effet conjoint d'une restriction alimentaire et hydrique sur la reproduction du lézard vivipare. Ces deux facteurs peuvent avoir des effets synergiques en favorisant un stress physiologique au-delà du seuil allostatique étant donné nos résultats précédents sur les liens entre balance hydrique et énergétique. Nous avons donc réalisé une expérience qui est en cours d'analyse et ne fait pas partie des chapitres de cette thèse, mais dont je vous présente rapidement

les enjeux. Nous avons suivi des femelles adultes *Zootoca vivipara* depuis le début jusqu'à la fin de la gestation en leur imposant un traitement nutritif différencié (nourriture *ad libitum* ou nourriture limitée) couplé à un traitement hydrique différencié (eau *ad libitum* ou restriction hydrique modérée). Nous avons suivi l'effet de ces traitements grâce à des mesures morphologiques (masse, réserves caudales, épaisseur du muscle) et physiologiques (osmolalité, corticostérone, triglycérides) au cours de la gestation et juste après la mise-bas. Après la naissance, nous avons mesuré les effets des différents traitements sur le nombre de juvéniles, leur masse, leur morphologie, leur sexe, et sur leurs pertes hydriques totales. Grâce au dispositif d'enclos semi-naturels au CEREEP-Ecotron IleDeFrance, nous avons pu mesurer l'effet sur la survie et la croissance à 2 mois (après une saison d'activité) puis à 1 an (après une première hibernation). Les premiers résultats indiquent un effet marqué du manque d'eau sur la prise de masse au cours de la gestation mais peu d'effet de la restriction alimentaire (Figure 2A). A l'inverse on observe un effet plus important du manque de nourriture sur la différence de masse perdue lors de la mise bas entre les traitements, c'est-à-dire sur l'investissement dans la reproduction (Figure 2B). Des effets synergiques semblent jouer sur le succès reproducteur (nombre de jeunes vivants à la naissance divisé par le nombre d'œufs initialement produits). Le succès reproducteur moyen est globalement faible pendant l'expérience et semble affecté par l'effet synergique des deux contraintes alors que l'une seulement ne modifie pas le succès si on compare aux femelles du groupe contrôle (Figure 3).

**A****B**

**Figure 2:** Boxpot représentant (A) le gain de masse au cours de la gestation et (B) la perte de masse liée à la mise bas ; chez des femelles ayant subi lors de la gestation une restriction hydrique (en bleu), une restriction alimentaire (en beige) ou la combinaison des deux (en orange), comparé à des femelles ayant eu de l'eau et de la nourriture *ad libitum* (en vert).





**Figure 3 :** Moyenne et intervalle de confiance du succès reproducteur des femelles ayant subi lors de la gestation une restriction hydrique (en bleu), une restriction alimentaire (en beige) ou la combinaison des deux (en orange), comparé à des femelles ayant eu de l'eau et de la nourriture *ad libitum* (en vert). Cette moyenne est calculée pour minimum 30 femelles par groupe.

Ces premiers résultats semblent montrer que la restriction conjointe d'eau et de nourriture a des effets immédiats sur le résultat de la reproduction, probablement dus au compromis d'allocation des ressources entre la maintenance de la balance hydrique et énergétique de la mère et l'investissement reproducteur. Des analyses complémentaires sur les traits morphologiques liés à la mobilisation de réserves en réponse à ces restrictions (réserves caudales, muscles) et sur la survie des jeunes à 2 mois et 1 an nous permettront d'évaluer l'impact précis de ces restrictions sur la valeur sélective des mères. Ces effets sont susceptibles d'impacter la dynamique de populations des lézards dans les zones où les précipitations vont diminuer et où les espèces d'invertébrés sont menacées.

## 5) Améliorer la prise en compte des effets de la balance hydrique sur la valeur sélective dans les modèles mécanistiques

Des modèles robustes sont actuellement développés afin d'évaluer la vulnérabilité des organismes vivants aux changements climatiques. Des approches mécanistiques, dérivées de la théorie métabolique de l'écologie, ont été proposées pour modéliser le budget énergétique d'un organisme en réponse à des variations des conditions climatiques et aux stratégies physiologiques et comportementales (Kearney & Porter, 2009). Ces modèles ont besoin de données éco-physiologiques pour comprendre comment la stratégie alimentaire, la disponibilité de la nourriture ou encore l'habitat interagissent avec les budgets de chaleur, d'eau et d'énergie tout au long du cycle de vie. Ainsi, Kearney *et al.* (2013) mettaient en évidence l'importance de l'équilibre hydrique chez les ectothermes terrestres et soulignaient le besoin de combler les lacunes dans notre compréhension des traits physiologiques et comportementaux qui affectent l'équilibre hydrique par rapport à notre connaissance des traits thermiques. Les résultats présentés dans cette thèse couplés à de nombreuses autres études de ces dernières années (Rozen-Rechels *et al.*, 2019) servent à améliorer la compréhension des comportements d'hydrorégulation chez les squamates et leurs impacts sur les niches climatiques exploitables dans les résultats des modèles de distribution d'espèces (Kearney *et al.*, 2018). De façon générale, pour pouvoir prédire comment les espèces vont répondre aux changements environnementaux futurs et gérer ces réponses, il est nécessaire de caractériser précisément la manière dont les organismes interagissent avec leur environnement et les mécanismes biologiques qui sous-tendent ces interactions. Briscoe *et al.* (2022) soulignent le potentiel des modèles biophysiques pour prédire les limites de distribution d'espèces basées sur les bilans d'énergie et d'eau, en tenant compte du comportement et de la phénologie. Les auteurs identifient le manque de données disponibles comme un défi actuel limitant l'application généralisée des modèles biophysiques. En effet, ces modèles basés sur les processus nécessitent beaucoup plus de données éco-physiologiques que les modèles corrélatifs mais sont supposés être plus robustes (Ceia-Hasse *et al.*, 2014). Les données récoltées dans le **Chapitre 2** ont par exemple servi d'entrées pour modéliser les probabilités d'extinction de populations de lézards vivipares dans le Massif central en fonction de la disponibilité en eau avec l'outil NicheMapR (Kearney & Porter, 2020, travaux en cours d'Andréaz Dupoué). L'ajout dans le modèle d'une stratégie d'hydrorégulation optimale (supposant une capacité des lézards à choisir des abris limitant leurs pertes hydriques évaporatives) a permis d'améliorer les performances des modèles mécanistiques et de mieux prédire les distributions de cette espèce dans le Massif Central. Prendre en compte les compromis comportementaux avec les prédateurs pourrait

également améliorer les prédictions sur les distributions futures en fonction de la variation spatiale du risque de manque d'eau et des pressions de prédation.

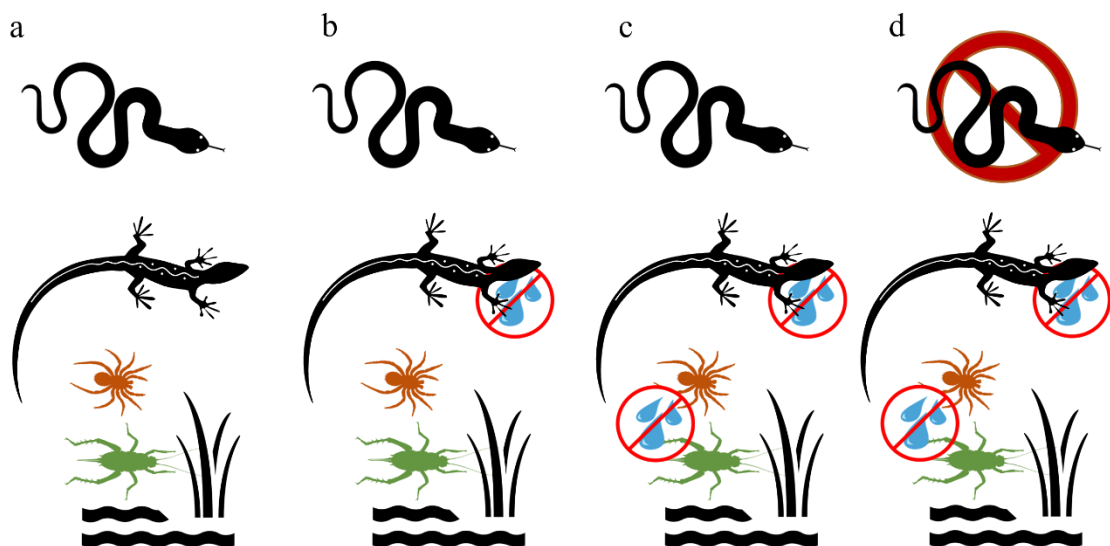
#### **6) Améliorer la prise en compte des relations biotiques**

Il est par ailleurs admis en écologie des communautés que les changements climatiques vont modifier les interactions biotiques entre espèces, notamment les relations avec les proies ou les prédateurs (Tylianakis *et al.*, 2008; Gilman *et al.*, 2010; Bretagnolle & Terraube, 2019). Ces modifications peuvent être la conséquence indirecte des ajustements comportementaux ou physiologiques de chaque espèce et donc affecter le risque de prédation (Miller, Matassa & Trussell, 2014) et le comportement de nourrissage (Levy *et al.*, 2016). La prise en compte des effets biotiques dans la distribution des espèces est donc aussi un défi majeur de ces dernières années, les études étant de plus en plus nombreuses à souligner l'importance de relations de prédation ou de compétition dans la vulnérabilité des espèces aux changements globaux (Tylianakis *et al.*, 2008). Les rôles respectifs des effets biotiques et abiotiques dans la régulation des populations lorsqu'ils sont susceptibles d'avoir des effets opposés sont aussi à prendre en compte pour développer une nouvelle génération de modèles (Voigt, Perner & Hefin Jones, 2007; Berggren *et al.*, 2009). Nos résultats sur les compromis entre évitement des prédateurs et balance hydrique sont donc importants pour comprendre les mécanismes biotiques qui peuvent jouer de concert avec les contraintes climatiques. Par exemple nos travaux suggèrent que les populations susceptibles d'être affectées par la diminution des précipitations ne réagiront pas de la même manière s'il y a par ailleurs une forte pression de prédation ou pas. Lors de la mise en place de plans de conservation d'espèces, il est important d'intégrer ces contraintes synergiques pour évaluer la vulnérabilité des populations. Les coûts de la chémoréception sont d'autant plus importants à étudier que celle-ci n'est pas seulement utilisée par les animaux terrestres pour l'évitement des prédateurs mais aussi pour la recherche de nourriture et de partenaires sexuels (Mathis & Crane, 2017; Bels & Russell, 2019), deux éléments essentiels à la valeur sélective des individus.

#### **7) Extension de l'approche à l'échelle de la communauté**

Ces résultats obtenus à l'échelle individuelle en conditions contrôlées de laboratoire ne sont pas toujours représentatifs de ce qu'il se passe dans les écosystèmes. Ces conditions artificielles omettent une partie de l'environnement biotique naturel des lézards (tels que les parasites, les conspécifiques) et le stress généré par le maintien en captivité peut induire des biais dans les réponses physiologiques et comportementales des animaux (Greenberg, 1992; Burghardt, 2013; Mikó *et al.*, 2015). Des expériences complémentaires, par exemple en mésocosmes dans un

environnement plus réaliste, ou encore des études corrélatives dans des populations naturelles, sont souhaitables pour approfondir ces connaissances. Par exemple, nous proposons une expérience en mésocosmes pour étudier l'impact de la disponibilité de l'eau à différents niveaux de la chaîne trophique, afin de vérifier si les compromis entre balance hydrique et relations trophiques mis en évidence pendant cette thèse sont maintenus (Figure 4). Cela pourrait permettre aussi de mesurer l'effet d'un risque de prédation sur la cascade trophique (Bestion *et al.*, 2015) dans le cas où l'eau serait une contrainte abiotique supplémentaire. Bestion *et al.* (2015) ont ainsi démontré que les effets indirects de la menace de prédation peuvent se propager jusqu'à affecter à la fois les herbivores et les plantes dans une chaîne alimentaire terrestre et soulignent la nécessité de quantifier l'importance de ces effets indirects dans les communautés naturelles. Nous avons vu que l'état hydrique peut affecter les comportements anti-prédateurs chez le lézard vivipare et il est donc possible que l'on trouve des résultats différents en ajoutant cette contrainte hydrique. On peut par exemple supposer que les lézards déshydratés seront moins sensibles à la présence d'odeur de prédateur (dû à l'impact sur la chémoréception) et qu'ils auront un effet top-down sur les proies et les producteurs primaires proche de celui observé en l'absence de prédateurs.



**Figure 4** : Proposition d'expérience en mésocosmes pour tester les effets de la balance hydrique des différents niveaux trophiques sur les régulations top-down et bottom-up des écosystèmes. Les serpents représentent une menace de prédation pour les mésoprédateurs lézards, qui pourraient être ajoutés sous forme d'odeurs dans l'environnement (comparaison de (c) et (d) par exemple). L'accès à l'eau de boisson peut varier entre (a) accès pour toute la chaîne trophique, (b) uniquement pour les proies invertébrées et les producteurs primaires ou (c) pour aucun des animaux.

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## Remerciements

Je voudrais remercier sincèrement les nombreuses personnes qui ont participé au travail réalisé pendant cette thèse, mais aussi celles qui ont contribué à me mener jusque-là et qui ont partagé les bons moments de ces dernières années.

Tout d'abord, je remercie grandement mes deux encadrants, **Jean-François Le Galliard** et **Olivier Lourdais**, pour leur soutien, leur investissement et les connaissances qu'ils m'ont apporté pendant ces années de thèse. Merci **Jean-François** de m'avoir en premier lieu fait confiance pour mon stage de Master 2 en 2019. Nous nous sommes rencontrés lors du stage d'écologie de terrain de ma Licence 3 à Foljuif, pendant lequel j'ai eu la chance de tomber sur le groupe travaillant avec toi sur les lézards. Lorsque je suis venue te voir pendant mon Master pour discuter d'un stage et d'une thèse, tu m'as proposé un sujet avec Olivier et nous avons commencé notre collaboration scientifique. Merci de m'avoir aidé à co-construire un projet de thèse qui me plaisait pour obtenir une bourse à l'ENS, je suis heureuse d'avoir travaillé avec toi pour mes débuts dans la recherche. Merci d'avoir partagé tes connaissances de manière si pédagogue et passionnée, avec moi qui n'était pas une herpétologue à l'origine. J'ai été heureuse de partager également des moments sur le terrain ou à Foljuif où j'ai pris grand plaisir à discuter de plein de sujets, scientifiques ou pas, de la géopolitique au féminisme. Merci d'avoir éclairé nos matinées de travail en week-end avec des chocolaines, de nous avoir fait partager les bons légumes de ton potager et les caresses d'Ilùn. Merci **Olivier** pour ton implication dans ma thèse malgré la distance entre Chizé et Paris. J'ai toujours pris plaisir à venir te voir au CEBC, à discuter avec toi de tes nombreux projets scientifiques en cours et rencontrer tes autres étudiant·e·s. Merci pour ta débrouillardise pour construire des circuits respiro, pour ton humour, ta bonne humeur et tes références filmographiques à point nommé. Merci à vous deux d'avoir rendu cette expérience si enrichissante et agréable.

Je remercie également les membres de mon jury de thèse, **Dr. Loïc Teulier**, **Pr. Raoul Van Damme**, **Dr. Elvire Bestion** et **Pr. Christel Lefrançois** d'avoir accepté d'évaluer mon travail.

Ce travail a bénéficié de riches discussions lors de mon comité de suivi scientifique, et pour cela je remercie **George Bruschi**, **Julien Cote**, **Sandrine Meylan** et **Marion Valeix**.

J'ai eu la chance de faire partie de deux équipes issues de deux laboratoires, que je souhaite remercier pour leur accompagnement au quotidien.

A l'Institut d'Ecologie et des Sciences de l'Environnement de Paris (iEES Paris), je remercie les membres de l'équipe VPA (Variabilité Phénotypique et Adaptation) et particulièrement **Tom Van Dooren** : merci d'avoir été un chef d'équipe impliqué et d'avoir su maintenir le lien entre nous pendant le confinement ; merci à **Thomas Tully** et **David Laloi** pour nos discussions régulières. Un immense merci à **Sandrine Meylan** car bien que je ne fasse pas officiellement partie de ton équipe tu m'as toujours fait sentir que j'étais la bienvenue. La saison de terrain ne serait pas si agréable sans tes délicieux gâteaux, l'allégresse de Marco, ton enthousiasme permanent et nos papotages sur les trajets CEREEP-Kremlin. Tu es le modèle féminin qui manquait à mon encadrement officiel ! Un autre immense merci à **David Rozen-Rechels** dont j'ai sollicité les conseils avant même de m'engager dans cette thèse et bien des fois ensuite pendant ces trois années. Merci de me partager ton expérience de la vie avec honnêteté et de me montrer le chemin rêvé dans la recherche ! Je remercie aussi **Andréaz Dupoué** et **Arnaud Badiane**, les post-doc de l'équipe lézard, pour leur gentillesse et leur partage de connaissances (et de superbes photos dont celles dans ce manuscrit). Je remercie enfin tout particulièrement le personnel de la gestion de iEES Paris, pour leur efficacité sans pareil et leur gentillesse : merci **Carole Bousquet**, **Johana Azzi**, **Véronique Marciat** et **Julie Legoupi**. Merci aussi à **Martine Maibeche**, **Paola Paradisi** et **Pierre Federici** (sans oublier Frédérique depuis peu) pour votre efficacité à diriger ce laboratoire dans la bonne humeur. Merci également à **Anouk Pellerin** et **Baptiste Lemaire** pour leur aide au laboratoire lors de l'analyse de mes échantillons de plasma. Merci enfin à tous les membres permanents de iEES Paris avec qui j'ai pu échanger à la pause-café ou au pot du vendredi, en particulier Nicolas Loeuille, Julien Gasparini, Adrien Frantz, Clotilde Biard, Yoan Marcangeli, Thibaud Monnin.

Au Centre d'Etudes Biologiques de Chizé (CEBC), je remercie tous les membres de l'équipe **Ecophy** de m'avoir accueillie parmi vous lors de mes venues saisonnières. Merci aux jeunes chercheur·se·s sur place pour votre accueil toujours adorable, votre aide précieuse pour le transport et les clés de chambre, pour les repas au restaurant de la station : merci **Agathe Gaffard**, **Léa Lorrain**, **Matthias Renoirt**, **Marion Cheron**, **Pauline Bellot**, **Jean-Pierre Vacher**, **William Jouanneau**, **Thomas Duchesne** et les autres. Un merci tout particulier à **Mathias Dezetter**, mon co-thésard inversé entre Paris et Chizé, pour ta disponibilité, ta gentillesse et ton aide généreuse. Merci aux post-doc d'Olivier que j'ai eu la chance de côtoyer sur le terrain ou en manip : Thank you **George Bruschi** for being so enthusiastic when you discovered fieldwork in France and for your precious help in my latter project. Obrigada **Rodrigo Gavira** por a sua colaboração durante o meu projeto de Master ! Merci aussi à

**Michaël Guillon** pour son aide avec la cartographie et ses discussions intéressantes. Enfin merci à **Delphine Bonnet** d'avoir géré les aspects logistiques de mes venues au CEBC.

Un autre endroit où j'ai passé beaucoup de temps pendant ma thèse est le **CEREEP-Ecotron IleDeFrance** à Foljuif, et c'est peut-être même là qu'est née mon envie de faire une thèse en écologie évolutive lors d'une UE de L3. Aussi je souhaite remercier chaleureusement le personnel de la station et tout particulièrement **Béatrix Decencière** et **Simon Agostini**, pour leur aide à la capture et à l'élevage des lézards, et pour les déjeuners partagés au soleil après l'effort. Je remercie aussi le reste du personnel pour leurs invitations aux barbecues estivaux sur la station qui rendent la vie sur place bien plus agréable.

La majorité des travaux présentés dans cette thèse ont bénéficié de la précieuse aide de stagiaires que je remercie tout particulièrement pour leur implication, leur motivation et aussi leur bonne humeur dans les moments de vie quotidienne à la station ou au labo. Merci **Jérémy Lefèvre**, **Anna Kawamoto**, **Anna Marcout**, **Elsa Martin**, **Audrey Ely**, **Jules Domalain**, **Emilie Pen**, **Lolita Muller**, **Noé Bastard**, **Jules Paraskevopoulos**, **Matthias Colin**, **Lauryn Trouillot** et celles et ceux que j'ai croisé en 2022.

Mes travaux ont bénéficié du financement de l'ANR AQUATHERM - " Rôles de la régulation hydrique et thermique dans les réponses écologiques au changement climatique " que je remercie pour son soutien, ainsi que les personnes avec qui j'ai pu échanger au cours des réunions annuelles du projet, notamment **Jean Clobert** et **Pierre de Villemereuil**. Je remercie également l'association **Cistude Nature** et Matthieu et Maud Berroneau pour leur collaboration lors de notre étude de terrain.

Pendant ma thèse j'ai eu l'occasion d'enseigner à l'université de La Rochelle, et je remercie les enseignant·e·s chercheur·se·s qui m'ont fait confiance et m'ont guidé dans cette expérience : **Coraline Bichet**, **Nathalie Imbert** et **Michaël Airaud**.

Je remercie également mon école doctorale **Euclide** et **La Rochelle Université** de m'avoir acceptée dans leurs cursus, et de m'avoir octroyé une prolongation à mon contrat doctoral suite à la crise du Covid-19.

Je voudrais remercier très chaleureusement l'équipe des **membres non-permanents de iEES Paris**, qui ont fait de ce labo un lieu si agréable pour venir travailler, mais aussi pour les bons moments en dehors du labo, au pot du vendredi, aux JRJC ou en conférence, que nous avons partagés. Merci aux ancien·ne·s pour leur accueil à mes débuts : Lauren, Vincent, Romain,

Adam, Iry, Jérôme ; merci à Basile pour avoir vécu avec moi toutes les étapes de ces 3 années et demi de thèse, merci Alice, Antoine, Fatima G I, Léna, Youssef ce fût un réel plaisir de vous côtoyer et de discuter de la vie avec vous ; merci aussi à Arthur, Amandine, Marie B, Corentin, Fatima E M, Marie G, Samuel, Paul, Aurélie, Théo, Souleyman, Hajar, Gabin, Pete, Matilda, Charles ; et merci à celles et ceux qui prendront la suite pour maintenir cette bonne ambiance : Antoine, Théo, Elsa, Nicolas P, Nicolas B, Romain.

Ces années de thèse ne se seraient pas si bien déroulées sans mes précieux·ses ami·e·s de l'ENS, alors un immense merci aux **Bio15** pour leur soutien, nos discussions scientifiques ou pas à la montagne, nos week-ends aux Taillis, nos soirées au KB, les pauses estivales en Bretagne, en Ardèche et en Espagne, les manif et les cinés ! Merci merci à Clara, Thomas, Corentin, Alice, Louise, Yasmine, Auriane, Etienne, Romain. Merci à mes camarades hors de la biologie pour nos discussions sur le reste du monde : Cata, Cécile, Léa, Julien, Hélène, David, Isma et merci à Nina et Chloé d'avoir suivi mes aventures depuis Toulouse. Merci aux **Bio14** qui m'ont guidée et encouragée jusque-là, en particulier Benoît, Benjamin, Félix, PA et Sara.

Merci aux politiques qui défendent l'enseignement supérieur, l'université publique et la recherche fondamentale et qui nous permettent de garder espoir pour notre futur.

Merci aussi à tous les membres de l'éducation nationale et de l'enseignement supérieur qui m'ont mené jusque-là, de mon institutrice de CM2 qui nous a fait disséquer un cœur de mouton et m'a en tout premier émerveillée pour la biologie, à tou·te·s celles et ceux qui ont suivi, enseignant les sciences pour lesquelles iels ont su me passionner et me convaincre que j'avais ma place dans cette voie. Merci aussi à ma tutrice à l'ENS **Hélène Morlon**, à mes encadrant·e·s de stages précédents qui m'ont initié à la recherche et donné envie de faire cette thèse, **Mathieu Molet, Michael Nachman, Shelly Masi** et **Laure Ségurel**.

Pour finir je voudrais remercier ma famille (ou presque) :

**Anto**, rien n'aurait été pareil sans toi à mes côtés. De nos confinements dans notre étriqué royaume vert ou dans les grands espaces des Taillis, de nos vacances dans les superbes pays méditerranéens ou à la découverte de la fête foraine de Niort pendant mon séjour à Chizé, merci d'avoir toujours été là. J'espère que l'on vivra encore beaucoup d'aventures ensemble, là où la recherche nous mènera et ailleurs.

Merci infiniment à ma famille, à **mes parents** pour m'avoir toujours soutenue, m'avoir incitée à garder toutes les portes ouvertes et à faire avant tout ce qui me plaisait, peu importe les

débouchés derrière. Merci à mon brillant **petit frère** qui je l'espère suit une voie qui le satisfera autant que moi. Merci à celles et ceux qui ne sont plus là pour voir l'aboutissement de ces années d'étude et de travail, mais qui m'ont si bien accompagnée jusque-là et qui me manquent terriblement. A ma Mamijo à qui je souhaite avoir encore longtemps le plaisir d'expliquer mes recherches, et au reste de ma famille sur qui je sais que je peux toujours compter, en particulier Mumu, Juliette, Alice, Emma, Manu, Pierre, Arthur et Thierry.



## Autres activités réalisées pendant la thèse

### ***Participation à des congrès scientifiques***

- 2021 (Présentation orale) – Journées scientifiques de iEES Paris, Sorbonne Université Paris, “*Role of feeding in the water balance of a generalist ectothermic mesopredator*”
- 2021 (Poster) – Ecology across borders – Meeting of the British Ecological Society (BES) and the French Society for Ecology and Evolution (SFE<sup>2</sup>), Liverpool UK, “*The role of feeding in the water balance of an insectivorous lizard*”
- 2022 (Présentation orale) – Ecology and Behaviour, Strasbourg, France
- 2022 (Présentation orale) - SFE<sup>2</sup> GFÖ EEF - International Conference on Ecological Sciences, Metz, France, “*Water deprivation interacts with chemoreception and anti-predator behaviors in a Lacertid lizard*”

### ***Encadrement de stagiaires***

Encadrement principal :

- Elsa Martin – M1 BEE Paris – Mi-Mars/Mi-Mai 2020
- Jules Paraskevopoulos – M1 BEE Toulouse – Juin-Juillet 2021
- Lauryn Trouillot – M1 BEE Rennes – Avril-Mai 2022
- Matthias Colin – 1<sup>ère</sup> année AgroParisTech – 1 jour par semaine (année 2021-2022)

Co-encadrement lors des mois de terrain et d’expérimentations au CEREEP Ecotron IleDeFrance :

- Printemps/Été 2020 : Audrey Ely (césure AgroParisTech), Jules Domalain (L3 Université de Rouen), Anna Marcout (M1 MNHN Paris)
- Printemps/Été 2021 : Lolita Muller (L3 Université de Lille), Noé Bastard (L2 Université Savoie Mont Blanc), Emilie Pen (L3 Université de Paris), Théo Bodineau (M2 Université de Strasbourg)

### ***Enseignements***

|              |                        |                                 |           |     |
|--------------|------------------------|---------------------------------|-----------|-----|
| Licence (TP) | La Rochelle Université | Physiologie animale et végétale | 2020-2021 | 16h |
|              |                        |                                 | 2021-2022 | 24h |

### ***Formations doctorales suivies***

- Formation à l’**expérimentation animale** sur faune sauvage, dispensée par le Muséum National d’Histoire Naturelle (MNHN), cours théoriques (mars 2021) et cours pratiques sur reptiles à la SETE Moulis (septembre 2021)

- Formation « Bonnes pratiques pour une **recherche reproductible** en écologie numérique » dispensée par le Cesab (Centre de synthèse et d'analyse sur la biodiversité, programme de la fondation pour la recherche sur la biodiversité) – Montpellier (décembre 2019)
- Formation « La **phylogénie** : support à l'analyse de l'évolution » dispensée par le Muséum National d'Histoire Naturelle (MNHN Paris) – Paris (mars 2020)
- Formation « Environnement numérique : **Visualisation des données** sous R » dispensée par Sorbonne Université (janvier 2020)
- Participation au **Colloque des doctorant** de l'ED Euclide, organisé par La Rochelle Université (septembre 2021)

### *Participation à d'autres projets de l'équipe*

- **Optimising hydroregulation to restore water balance and alleviate climate stress in a dry-skinned ectotherm** *Andréaz Dupoué, Pauline Blaimont, Urtzi Enriquez-Urzelai, Michaël Guillon, Olivier Lourdais, Arnaud Badiane, Chloé Chabaud, Sandrine Meylan, Alexis Rutschmann, David Rozen-Rechels, Donald B Miles, Fabien Aubret, Jean Clobert, Murielle Richard, Beatriz Decencièrre, Simon Agostini, Michael R Kearney, Barry Sinervo, Jean-François Le Galliard* – en cours d'écriture
- **Influence des ressources hydriques et nutritives sur les stratégies de thermo-hydrorégulation du lézard vivipare en mésocosmes** – *Expérience réalisée pendant le stage d'Audrey Ely et analyses conduites pendant le stage de master 2 de Théo Bodineau* – valorisation à venir

### *Vulgarisation et diffusion de connaissances*

Interview pour un dossier sur les lézards dans la Revue Salamandre, paru dans le numéro 264 de juin-juillet 2021

Animation du compte Tweeter de vulgarisation « la Bio au Labo » pendant une semaine en mai 2021 (<https://labioaulabo.tumblr.com/>)

Podcast sur les résultats du Chapitre 2 dans l'épisode 7 « Le journal de Tata Doc » animé par Elise Verrier, enregistré en novembre 2022, disponible le 21 mars 2023

Participation à l'organisation des journées d'études du Centre de formation sur l'environnement et la société (CERES, ENS-PSL) en souvenir de David Claessen : 2020 – 2021 - 2022

### *Activités collectives non scientifiques*

Février 2020 : Organisation de deux journées de retraite pour les jeunes chercheurs et chercheuses de iEES Paris (doctorant.e.s, postdoc, ATER) à la station d'écologie forestière de Fontainebleau

Mars 2021 – maintenant : élue représentante des doctorant.e.s au sein du conseil de laboratoire de iEES Paris

Avril 2021 – maintenant : Participation au comité SBEA (Structure Bien-être Animal) du CEREEP Ecotron IleDeFrance UMS 3194



## Influence de la balance hydrique sur les interactions trophiques et le risque de prédation chez un mésoprédateur ectotherme terrestre

### Résumé :

Les changements climatiques actuels impactent les températures environnementales mais également la disponibilité en eau dans les écosystèmes, ce qui peut perturber les interactions trophiques entre proies et prédateurs. Chez les ectothermes terrestres, il existe un conflit physiologique entre la régulation de la température corporelle et de la balance hydrique, mais nous manquons de données pour caractériser les effets des compromis associés sur les interactions biotiques. Cette thèse s'intéresse à cette problématique à travers l'étude écophysiologique et comportementale de traits liés à la thermo-hydrorégulation et aux relations trophiques chez le lézard vivipare (*Zootoca vivipara*). Ce mésoprédateur généraliste se nourrit d'une grande diversité de proies et est soumis à une forte prédation, notamment par des serpents spécialisés. Les reptiles présentent une grande diversité de traits fonctionnels associés à la thermo-hydrorégulation dont mes travaux caractérisent les déterminants individuels et géographiques chez le lézard vivipare. Ensuite, à l'aide d'approches expérimentales, je montre que la restriction en eau ne peut pas être compensée par l'alimentation chez cette espèce, mais que la nourriture exacerbe les conflits comportementaux entre thermo- et hydrorégulation. Par des observations au laboratoire, j'étudie également les compromis entre hydrorégulation comportementale et évitement des prédateurs. Je démontre que les comportements de détection des prédateurs par la chémoréception augmentent les pertes hydriques et qu'ils sont donc sensibles à la restriction en eau. Ces travaux suggèrent l'existence d'effets des stratégies comportementales de thermo-hydrorégulation sur les relations avec les proies et les prédateurs.

Mots clés : balance hydrique, alimentation, comportements anti-prédateurs, physiologie, ectotherme, thermo-hydrorégulation

## Influence of water balance on trophic interactions and predation risk in a terrestrial ectothermic mesopredator

### Summary:

Ongoing climate change is affecting environmental temperatures but also water availability in ecosystems, which may disrupt trophic relationships between prey and predators. In terrestrial ectotherms, there is a conflict between the regulation of body temperature and water balance, but we lack data to characterize the effects of the associated trade-offs on biotic interactions. This thesis addresses this issue through the ecophysiological and behavioural study of traits related to thermo-hydroregulation and trophic relationships in the Common lizard (*Zootoca vivipara*). This generalist mesopredator feeds on a wide variety of prey and is subject to heavy predation, especially by specialized snakes. Reptiles exhibit a large range of functional traits associated with thermo-hydroregulation, and my work characterizes the individual and geographic determinants of these traits in the Common lizard. Then, using experimental approaches, I demonstrate that water restriction cannot be compensated by food intakes in this species, and food actually exacerbates behavioural conflicts between thermo- and hydroregulation. Through laboratory observations, I also investigate the trade-offs between behavioural hydroregulation and predator avoidance. I show that detecting predators via chemoreception increases water loss and this ability is therefore sensitive to water restriction. This work suggests that the behavioural strategies employed by organisms for thermo-hydroregulation can influence the dynamics of predator-prey relationships.

Keywords : water balance, diet, anti-predator behaviours, physiology, ectotherm, thermo-hydroregulation



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