EX-SITU AND *IN-SITU* CONSERVATION IN PRACTICE: EFFECTS OF SPECIES-MANAGEMENT STRATEGIES ON THE BEHAVIOR AND MICROBIOTA OF AMPHIBIANS.

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To my grandparents,

for embracing and cultivating my love of nature,

for their inspiring patience and tenderness towards the world.

« […] c'est bien simple, tout ce que je défends, c'est la nature... Appelez cela comme vous voulez. Liberté, dignité, humanité, écologie... Cela revient au même. »

Romain Gary, in *Les Racines Du Ciel.*

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ABSTRACT

In the midst of the current biodiversity crisis, amphibians are the most endangered vertebrate class, with over a third of species globally threatened with extinction. Given the urgency of the situation and the impossibility to rapidly impede all threats putting them at risk, conservation instances recommend, among possible actions, to move amphibians from threatened locations to safer sites in the wild (*in-situ* translocations) or into captivity to build "survival assurance populations" that could prevent the extinction of threatened species and enable their latter reintroduction in the wild (*ex-situ* collections). While such human-driven interventions have undoubtedly contributed to the survival of several amphibian taxa, their success is still rather limited, and they often fail to ensure the persistence of sustainable populations. Management protocols are now adapted to overcome long-known limitations associated with translocations and captive breeding (*e.g.*, increased risks of disease spread, genetic bottlenecks, etc.) but many other, unexplored, aspects of the biology of amphibians may be affected by these conservation strategies, and could thus explain their mixed results. For example, several authors recently recommended to incorporate behavior and microbiota to amphibian conservation research since their consideration gave very promising results in other taxa. Indeed, the bacterial communities that reside on the skin of amphibians play a crucial role in their resistance to deadly pathogens and are strongly influenced by environmental factors. Therefore, the displacement of amphibians and their *ex-situ* management under husbandry protocols (which typically involve artificial cycles of temperature and habitat-shifts to induce breeding) could restructure their skin microbiota and deplete it from protective symbionts, thus increasing their vulnerability to disease. Moreover, amphibians could behaviorally adapt to the inherent homogeneity and predictability of captive environments; consequently, behavioral variation among- (personality) and within- (plasticity) individuals may decrease *ex-situ*. These dimensions of behavior have major

ecological implications, and the potential behavioral uniformization of captive amphibians and reduction of their behavioral plasticity could thus jeopardize reintroduction efforts.

In this context, my objectives were to determine whether common amphibian conservation practices such as *in-situ* translocation and *ex-situ* husbandry affected their skin microbiota, and to investigate whether long-term captivity had consequences on their personality and behavioral plasticity. I hypothesized that any displacements, whether into captivity or between wild sites, would disrupt the skin bacterial communities of amphibians. Moreover, I predicted that their behavioral variability would decrease *ex-situ*. To test these hypotheses, I established an *ex-situ* collection of newts managed under typical protocols for survival assurance populations and monitored their activity, exploration and boldness for 10 months; I also repeatedly sampled their skin microbiota to characterize the effect of husbandry protocols on its structure using high-throughput sequencing. Moreover, I translocated salamander larvae between similar wild sites, using mesocosms to keep track of each individual; I sampled their skin microbiota before and 15 days after moving them to determine how *in-situ* translocations may affect their skin bacterial communities.

The results of my research suggest that *ex-situ* conservation is associated with a loss of diversity in both individual behavior and in amphibian skin microbiota, and that artificial habitat-shifts implemented as part of husbandry protocols strongly restructure their skin microbial communities. Indeed, among- and within-individual variation in behavior decreased throughout time in captivity, suggesting that the newts became more similar in their personalities and more predictable in their behavioral responses. Moreover, the richness and diversity of their skin bacterial communities decreased rapidly and lastingly after their displacement from the wild into captivity. While habitat-shift protocols caused strong species-turnovers in the microbiota of the newts, they did not bring it back to its initial, wild structure. Such diversity loss *ex-situ* could have detrimental implications for reintroduced individuals as

they may not behaviorally and microbially readapt fast enough to survive in the wild. Conversely, I found that *in-situ* translocations have little effect on the amphibian microbiota, suggesting a strong resilience of skin bacterial communities to environmental change. Interestingly, the development of the experimental larvae strongly affected the composition and diversity of their skin microbiota, indicating that ontogeny can be a much stronger driver of its structure than the environment – at least in young life stages. Therefore, *in-situ* translocations may be a softer conservation approach for amphibians and their microbiota, but more research is needed to determine whether this is also the case with animals in other developmental stages. Taken together, my results suggest that species-management strategies for conservation have differential effects on many aspects of the biology of amphibians, and that their implications on the fitness of animals and the long-term success of conservation efforts should be investigated further. In conclusion, this work demonstrates the pertinence of integrating behavior and microbiota to amphibian conservation, and more generally, of adopting a comprehensive approach when evaluating species-management strategies. I hope my thesis underlines the necessity to develop protocols for maintaining diversity *ex-situ* and opens new avenues for applied research in this field. Ultimately, expending our understanding of the impacts of conservation methods on amphibians' biology will likely provide key information to improve future management strategies and thus ensure the persistence of viable amphibian populations.

Keywords: amphibian conservation, holobiont, microbiota flexibility, personality traits, population translocation, survival assurance populations.

RÉSUMÉ

En cette période actuelle de crise de la biodiversité, les amphibiens forment la Classe de vertébrés la plus en danger d'extinction au monde, avec plus d'un tiers d'espèces menacées. Compte tenu de l'urgence de la situation et de l'impossibilité d'éliminer rapidement toutes les menaces qui s'abattent sur eux, les autorités de conservation recommandent de déplacer les amphibiens se trouvant sur des sites menacés vers des sites naturels mieux protégés (translocations *in-situ*) ou en captivité, afin d'établir des collections d'« assurance-survie », permettant d'éviter l'extinction des espèces les plus menacées et d'éventuellement les réintroduire dans la nature ultérieurement (collections *ex-situ*). Bien que de telles interventions aient contribué à la survie de plusieurs taxons d'amphibiens, leur succès est encore assez limité et souvent elles échouent à assurer la persistance de populations durables dans le temps. Les protocoles de gestion des espèces sont désormais adaptés pour limiter les inconvénients les plus connus associés aux translocations et à la captivité (tels que le risque accru de propagation de maladies, les goulots d'étranglement génétique, etc.), mais de nombreux autres aspects inexplorés de la biologie des amphibiens pourraient être affectés par ces stratégies de conservation, et ainsi expliquer leurs résultats mitigés. Par exemple, plusieurs auteurs recommandent de prendre en compte le comportement et le microbiote dans la recherche sur la conservation des amphibiens, puisque cette approche s'est révélée fructueuse avec d'autres taxons. En effet, les communautés bactériennes qui résident sur la peau des amphibiens jouent un rôle crucial dans leur résistance aux pathogènes et sont fortement influencées par l'environnement. Par conséquent, le déplacement d'amphibiens et leur gestion en captivité avec des protocoles d'élevage (qui incluent généralement des cycles artificiels de température et de changements d'habitat pour stimuler la reproduction) pourraient restructurer leur microbiote cutané et en éliminer les bactéries symbiotes protectrices contre les pathogènes, augmentant ainsi leur vulnérabilité aux maladies. De plus, le comportement des

amphibiens pourrait s'adapter à l'homogénéité et à la prévisibilité qui sont inhérentes aux environnements captifs : par conséquent, la variation comportementale entre les individus (personnalité) et au sein de chaque individu (plasticité) pourrait diminuer *ex-situ*. Ces dimensions du comportement ont des implications écologiques majeures, et la potentielle uniformisation du comportement des amphibiens captifs ainsi que la réduction de leur plasticité comportementale pourraient ainsi compromettre les efforts de réintroduction.

Dans ce contexte, mes objectifs étaient de déterminer si les pratiques courantes de conservation des amphibiens (telles que la translocation *in-situ* et la captivité) affectent leur microbiote cutané, et d'étudier si la captivité à long terme a des conséquences sur leur personnalité et leur plasticité comportementale. J'ai émis l'hypothèse que tout déplacement d'amphibiens, qu'il soit vers la captivité ou entre sites naturels, perturberait leurs communautés bactériennes cutanées. De plus, j'ai prédit que leur variabilité comportementale diminuerait *ex-situ*. Pour tester ces hypothèses, j'ai fondé une collection *ex-situ* de tritons gérés selon des protocoles typiques pour les populations d'assurance-survie et j'ai mesuré leur activité, leur exploration et leur courage pendant 10 mois ; j'ai également échantillonné à plusieurs reprises leur microbiote cutané pour caractériser l'effet des protocoles d'élevage en captivité sur la structure de leurs communautés bactériennes cutanées en réalisant un séquençage à haut-débit. J'ai aussi transféré des larves de salamandres entre plusieurs sites naturels en utilisant des mésocosmes pour suivre chaque larve individuellement. J'ai prélevé leur microbiote cutané avant et 15 jours après leur déplacement pour déterminer comment les translocations *in-situ* affectent leurs communautés bactériennes cutanées.

Les résultats de mes recherches suggèrent que la conservation *ex-situ* est associée à une perte de diversité à la fois dans le comportement individuel et dans le microbiote cutané des amphibiens, et que les changements artificiels d'habitat mis en place dans le cadre de protocoles d'élevage en captivité restructurent fortement leurs communautés bactériennes cutanées. En effet, la variabilité comportementale de chaque individu et entre individus diminua fortement au cours de la captivité, suggérant que les tritons sont devenus plus prédictibles dans leurs réponses comportementales, et leurs personnalités plus similaires. De plus, la richesse et la diversité de leurs communautés de bactéries cutanées ont rapidement et durablement diminué après leur introduction en captivité. Malgré sa restructuration importante engendrée par les protocoles de changements artificiels d'habitat, le microbiote des tritons captifs ne retrouva pas une structure similaire à celle observée chez les individus sauvages. Une telle perte de diversité *ex-situ* pourrait avoir des conséquences néfastes pour les individus réintroduits si leur comportement et leur microbiote ne se réadaptent pas assez rapidement pour assurer leur survie dans la nature. À l'inverse, les translocations *in-situ* semblent avoir peu d'effet sur le microbiote des amphibiens, ce qui suggère que les communautés bactériennes cutanées peuvent être résilientes aux variations environnementales. La composition et la diversité du microbiote cutané des larves ont plutôt été affectées par leur développement, ce qui indique que l'ontogénèse peut être un déterminant plus fort de la structure du microbiote que l'environnement − du moins chez les individus aux stades larvaires. Par conséquent, les translocations *in-situ* pourraient être une approche de conservation plus douce pour les amphibiens et leur microbiote, mais des recherches supplémentaires sont nécessaires pour déterminer si ces conclusions s'étendent à tous les stades de développement. Dans leur ensemble, mes résultats suggèrent que les stratégies de gestion des espèces pour la conservation ont des effets variés sur de nombreux aspects de la biologie des amphibiens, et que leurs conséquences sur la viabilité des animaux et le succès à long terme des efforts de conservation devraient être étudiées plus en détail. Pour conclure, ce travail démontre qu'il est pertinent d'intégrer le comportement et le microbiote à la conservation des amphibiens, et plus généralement, d'évaluer les stratégies de gestion des espèces avec une vision plus globale. J'espère que ma thèse mettra en lumière la

nécessité de développer des protocoles pour maintenir plus de diversité *ex-situ* et ouvrira de nouvelles pistes pour la recherche appliquée dans ce domaine. L'approfondissement de notre compréhension des impacts des méthodes de conservation sur la biologie des amphibiens fournira probablement des informations cruciales pour améliorer les futures stratégies de gestion des espèces et ainsi assurer la persistance de populations d'amphibiens viables à long-terme.

Mots clés : conservation des amphibiens, flexibilité du microbiote, holobionte, populations d'« assurance-survie » traits de personnalité, translocation de populations.

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