

Secretomes and microbiomes of caecilian skin

PI: Mark WILKINSON

The Natural History Museum, UK

Abstract: The skin of frogs and salamanders is known to both harbor ecosystems of microorganisms that differ from the surrounding environment and which play a role in defence against pathogens, and also to produce secretions that contain bioactive molecules (such as antimicrobial peptides) that play an additional key role in the innate immune system. Some of these antimicrobial peptides are of pharmaceutical importance. In contrast, nothing is known about the skin secretions or skin microbiome of caecilians (Gymnophiona) – the third major extant group of amphibians. Background knowledge, especially about the role of caecilian skin in providing nutrition to hatchlings in some species, suggests that caecilian skin might be of particular interest. Building upon our expertise in caecilians (especially of French Guiana) and our very recently generated, unpublished, preliminary transcriptome and mass spectrometry data that demonstrate the presence of potentially antimicrobial peptides in caecilians, we propose to carry out new, innovative research into the caecilian skin secretome and microbiome. This project will collect samples of caecilian amphibians of French Guiana from a variety of life history stages, species and localities, in order to: 1) for the first time discover and characterize bioactive molecules in the skin secretions of wild caecilians; 2) determine how caecilian skin secretions vary inter- and intraspecifically, especially with respect to different life history stages and reproductive modes; 3) document for the first time the microbiome of caecilian skin; 4) determine inter- and intraspecific variation in the caecilian skin microbiome and how this correlates with the skin secretome; 5) seek substantial funding for future integrative analyses of the function and evolution of caecilian skin through transcriptomic, lipidomic, morphological and eDNA approaches.

Estimating the effects of climate change on calling behavior of tropical amphibians

PI: Elodie Courtois & Diego Llusia

University of Antwerp & LEISSA, Cayenne & MNHN Sorbonne University, Paris

Abstract: Climate change is incontestably going to be a major threat for biodiversity in the coming century. Its effects may vary geographically and are expected to differently affect taxa with respects to the species capacities to cope with environmental changes. Amphibians are currently the most threatened group of vertebrates and are ectotherms and use cutaneous respiration? Moreover, tropical amphibians seem to be particularly vulnerable to the current climate change in comparison with amphibians from other latitudes. The proposed project aims to examine amphibian vulnerability to climate change through the use of new tools such as acoustic monitoring, signal processing and big data analysis. Calling behavior plays an important role in sexual selection and reproductive success of amphibian anurans and it is strongly dependent of both temperature and hydric parameters. However, few studies have been focused on quantifying and qualifying the impact of climate change on such a key and environment-dependent behavioral trait in tropical anurans. Using automated sound recorders and environmental data loggers, this study proposes to monitor calling behavior in populations located at the thermal and hydric extremes of the geographic range of two tropical anurans, *Pseudis paradoxa* and *Hypsiboas raniceps*. This acoustic monitoring program will determine, by applying innovative signal recognition algorithm, the temporal pattern of calling activity of the study species across climatic gradients, and subsequently, the thermal and hydric breadths of their reproductive calling behaviour. The information provided by this study will be used to predict the response capacity of these species to deal with climate change, addressing an urgent and challenging

question in thermal and behavioural ecology of tropical amphibians. Additionally, with the support of a previous study addressing a similar question in European anuran species, the project will allow comparing the responses of tropical and temperate zone anurans and to identify conservation priorities and recommendations for this globally endangered species group, helping in the mitigation or prevention of the climate change impacts on biodiversity.

Dysbiosis Induction in *Aedes aegypti* to reduce Virus Transmission Efficacy: from natural microbiome diversity to lab experiments

PI: Isabelle Dusfour

IPG, Guyane

Abstract: This project gathers six experts in the fields of entomology, microbial ecology and biogeochemistry from Guyana and Canada to conduct a high-impact, holistic investigation of *Aedes aegypti* holobiont thriving in anthropogenic environments and provoking increasing arboviruses epidemics. Two experimental approaches, combining field and laboratory populations of *Ae. Aegypti*, will be used to demonstrate the deterministic effect of microbiome structure of larvae and adults on insecticide resistance and life history traits. Firstly, a field survey will be performed to characterize the bacteriome and mycobiome of natural *Ae. aegypti* populations. This result will be analyzed to infer the impact of biotic and abiotic features of water breeding sites on the structure of *Ae. Aegypti* microbiota and identify bacterial and fungal species whose distribution is related to the phenotypes. A genomic barcode to predict the selection pressure of breeding sites on *Ae. Aegypti* populations with one or another phenotype will be developed. Using synthetic microbial consortia, the second objective is aimed at evaluating the impact of microbiota dysbiosis on the mosquito population's resistance and larval history traits. Four tasks will be developed to reach the objectives: (i) field survey in 100 breeding sites selected in various anthropogenic environments, (ii) physicochemical characterization of breeding sites, (iii) characterization of bacterial and fungal communities in water and mosquitoes of each prospected sites, (iv) relation between the composition of breeding site and *Ae. Aegypti* microbiome, insecticide resistance level and life history traits and (v) development of dysbiosis experiments to alter phenotypes cited before. We expect a list of most important abiotic and biotic drivers affecting *Ae. aegypti* microbiomes, molecular barcode indicators for insecticide resistance and some of the vector capacity parameters of *Ae. aegypti* and the evaluation of dysbiosis treatments as an effective approach contributing to reducing the risk of virus transmission.

The influence of fungal networks on interactions among adult trees and seedlings

PI: Irene Ramirez Rojas

University of Antwerp, Belgium

Abstract: Tropical rainforests are the most diverse and productive ecosystems on Earth. These systems account for two-thirds of global plant diversity, and are often referred to as hyperdiverse in terms of tree species richness. Typically, any random draw of 300 adult individuals will represent over 100 different species. Mycorrhizal fungi act as a major conduit of carbon into soil, and affect competition between trees through connecting them belowground. This plant-fungal interaction is one of the most abundant symbioses on earth and is tightly linked to plant nutrient limitation. Forests in French Guyana are unique in being among the most pristine and well-studied lowland tropical rain-forests on Earth. In this project, we will test the effect of neighbouring trees on seedling success through mutualists and antagonists. While it is known that plants can connect to a "common mycorrhizal network", whether this occurs in the field and whether this affects seedling success

through increasing access to nutrients, carbon, and reduces vulnerability to antagonists is unknown. Moreover, whether this connection is dependent on whether the adult tree is of the same species is also, unknown, but may be an important driver of forest species composition through determining seedling success. Here we will manipulate connection to a common mycorrhizal mycelium and determine the effects on seedling growth depending on adult matching (same – other), as well as the effects on microbes colonizing seedling roots. This experiment will greatly increase our understanding of the importance of the mycorrhizal for seedling performance. This knowledge will allow better understanding of the importance of plantfungal relationships on seedling success and plant biodiversity.

Infectious disease and individual contributions to connecting ecological networks

PI: Christophe Thebaud & Javier Pérez-Tris
EDB, Toulouse & Complutense University of Madrid, Spain

Abstract: Ecological networks shape the wireframe that supports biodiversity, as network architectural properties such as complexity, connectance, compartmentalization or nestedness may underlie ecosystem stability and resilience. The last decade has witnessed an important development of network theory and methods to describe network properties. These developments have paved the road for question-driven approaches to network description. For example, recent research has unveiled the dynamic nature of network microscopic properties (such as species linkage level and strength or partner interdependencies changing both in space and time), yet we are far from understanding the extrinsic factors that may affect network architecture. Importantly, previous research has focused on species (rather than on individuals) as basic interactors in networks, thereby assuming random variance within each species in the individuals' propensity to interact with the different partners of that species. However, non-random variation in individual state may promote different links being disproportionately favoured by individuals of the same species. For example, in mutualistic networks such as those linking plants with pollinators and seed dispersers (which interact with plants to obtain nutritional rewards), individual nutritional requirements may create biased preferences for particular food sources, which may have consequences on the involvement of different individuals in connecting networks. We will explore this type of extrinsic influences on network properties by analysing the effect of infectious diseases (avian malaria and virus infections) on species linkage level and strength. We predict that diseased birds may favour distinct sets of plant species, arguably those which incorporate nutrients (in nectar or fruits) important for self-maintenance in the face of infection. Consequently, diseased birds will show stronger dependency on these plants, which reciprocally will gain control of pollination or seed dispersal services of a substantial part of the network. If microscopic network properties change in such a predictable way depending on which individuals are considered to compute network metrics, then the architecture of mutualistic networks would depend on pathogen prevalence, an overlooked mechanism to explain pathogen impacts on ecosystem functioning with important implications in ecology and conservation biology.

Microbiome dynamics of *Drosophila* in the amazon and their changes upon lab selection

PI: Wolfgang J. Miller
Medical University of Vienna, Austria

Abstract: Bacteria and viruses are the main compounds of the microbiome, which is an integral part

of all living organisms affecting host fitness and fecundity in many ways, ranging from their reproduction, physiology, nutrition, intra- and interspecies competition as well as pathogen protection and even speciation. They also can impact the success of alien invasive species when spreading throughout populations of indigenous species in nature, thereby massively harming local biodiversity. Although the complexity and dynamics of the microbiome are best studied in diverse model systems under laboratory conditions, we currently lack information on their composition and prevalence in the wild. In this proposal we aim to census and compare the microbiome of native versus invasive *Drosophila* species at different well-defined localities of French Guiana by means of NGS metagenomics. By this approach we aim to answer the following questions: How much do microbiota differ between invasive and native *Drosophila* hosts, do they reciprocally exchange their microbiota in contact zones, and finally what are the phenotypic consequences of such symbiont exchanges on fitness of natives and invaders? Moreover, when lines are transferred from their natural environment and domesticated to laboratory conditions, how much do they change in their microbial diversity and how long will it take them to adapt?

Variation in the diversity of parasite community between tropical and temperate bird species, and its relationship to host life history and ecology

PI: Jérôme Moreau

Biogeosciences, University of Burgundy, Dijon

Abstract: Tropical birds exhibit very different life history strategies (slow life histories: long-lived species, maturing late and producing fewer offspring during each reproductive event) in comparison to birds from temperate areas (fast life histories). Identifying the factors that govern these ecological differences has been a major research focus in evolutionary ecology. Parasites exert an important selective force on their hosts, affecting their behaviour and ecology. It has been hypothesized that organisms with slow life histories (tropical birds) should invest more in costly immune defences due to a greater exposure to parasites and a concomitant decrease in immunosuppressive hormones such as testosterone. Under this scenario, it has been expected that birds are exposed to a greater parasite pressure in tropical than in temperate areas. Most studies to date have produced contrasting results, perhaps due to the fact that they were focused on the effect of a single type of parasite (i.e. blood parasite) in a single area. However, any host harbours a community of parasites that influence one another's impact on its immune response, and parasite prevalence may be variable at different locations. In addition, host parasite communities are determined by a wide variety of factors including host ecology and life history. Determining how each of these factors controls parasite diversity and how their interactions shape parasite communities is a major topic in ecological parasitology. In order to improve our understanding of these processes, we need to simultaneously explore ecological and life history correlates of parasite diversity and abundance within hosts. This project therefore aims to elucidate whether (i) there is a general pattern for tropical birds to have higher parasite load and prevalence than temperate birds and (ii) the same ecological factors govern parasite diversity across different geographical areas. To this aim, we will organize fieldwork in French Guyana and in France, in two locations. Birds will be mist-netted and sampled for standard immune indices and parasites (blood parasites, ectoparasites and intestinal parasites) in a wide phylogenetic range of bird species with contrasted life history traits in both tropical and temperate regions. We expect that the data gathered during this project will significantly add to our understanding of the ecology of parasite communities within hosts, and of the evolution of host-parasite interactions and host life histories.

Is the bony-tongue fish *Arapaima* (Teleostei, Osteoglossiformes) really a monotypic genus?

PI: Christelle Tougard
ISEM, Montpellier

Abstract: *Arapaima gigas* (Schinz, 1822) is the only freshwater fish of the Neotropics that is listed as an endangered species in CITES, Appendix II. The main threat for *Arapaima* remains overfishing to commercialize meat for consumption. A lesser-known threat is translocation of specimens by aquaculture enterprises, a process that threatens to homogenize the genetic pool and even possibly extirpate locally adapted races or species. To facilitate management and conservation programs, several studies investigated the genetic diversity and population structure of *Arapaima* at various geographical scales using mitochondrial or nuclear DNA markers. These studies provided contradictory results: either (1) *Arapaima* forms a panmictic population with extensive genetic exchange among localities through the Amazon basin or (2) *Arapaima* is characterized by several Evolutionarily Significant Units. In contrast, the phylogeny of *Arapaima* was never investigated because *Arapaima* has long been considered to be a monotypic genus. A review of the early literature and holotypes stored in museums indicated however that, at least, four species of *Arapaima* should be considered valid. A fifth species, *A. leptosoma*, has also been discovered recently in the Solimões River, Brazil. Morphological differences among holotypes cannot simply be attributed to phenotypic plasticity. Better assessment of species diversity patterns is a critical conservation issue, especially for fishes threatened by overfishing such as *Arapaima*. In this context, our main objectives are: (1) to infer the phylogenetic relationships among *Arapaima* lineages, including holotype specimens (*A. mapae*, *A. gigas*, *A. leptosoma* and *A. arapaima*); (2) to delineate the species/taxonomic boundaries of *Arapaima* from morphology and molecular data. In order to limit our impact on wild *Arapaima* populations, only previously collected specimens now in museums and DNA or tissue samples (Brazil, Guyana and Peru) collected in the context of other projects will be integrated in our analyses. For the holotypes and museum samples, a protocol recently developed to build and sequence shotgun Illumina libraries from small quantities of degraded genomic DNA will be used. DNA extraction with blank controls will be performed in the Platform "ADN dégradé" of ISEM (LabEx CeMEB) dedicated to degraded DNA experiments. A similar protocol will be used for the other samples in a standard lab. Libraries will be sequenced on an Illumina HiSeq2000. Mapping of short reads (≤ 100 bp) and annotation of the complete mitochondrial genomes (or mitogenomes) and/or nuclear markers will be carried out against available reference genomes of *Arapaima*. Phylogenetic analyses will be conducted using a maximum-likelihood approach and Bayesian inference. Species delimitation will be investigated using three methods (PTP, bGMYC and BPP). We will compare this new, reliable molecular phylogeny and the species delimitation results with those from morphological analyses of the same specimens, and these analyses will allow us to broadly test the null hypothesis of monotypy which, if rejected, could support recent inferences of multiple species for the genus *Arapaima*. Because of its high economic, cultural and scientific value, the possible need to recognize several distinctive species of *Arapaima* in the Neotropics has huge implications for conservation, management and aquaculture.

POST-disturbance resilience of food WEBS and ecosystem multifunctionality along a gradient of simulated drought

PI: Céline Leroy & Régis Cereghino
Amap, Kourou & EcoLab, Toulouse

Abstract: Future climate scenarios forecast a 10-50% decline in rainfall in the north-eastern coasts of

South America and in central Amazonia. Yet, little is known of the mechanisms which ensure food web re-assembly after severe drought in “naïve” tropical ecosystems, and of the extent to which food-web responses mediate post-drought resilience of ecosystem functions beyond currently observed stress intensities. Taking advantage of a natural system that is small, contained, and widely distributed in neotropical forests (tank-forming bromeliads and their aquatic microbial-faunal food webs), we will emulate complete drying and then rewetting of this ecosystem in a field experiment. The effects of treatments (droughts) will range from current average conditions to extreme events, and will be evaluated in time against control bromeliads. We will follow-up core ecosystem functions (detrital mass loss, microbial respiration, photosynthetic activity of algae) and their potential drivers (fungal: bacterial biomass, predator: prey biomass ratio, autotrophic: heterotrophic microorganism biomass ratio), as well as ecosystem multifunctionality (the average of normalized functions). The relative importance of in situ resistance vs recolonisation by immigration will be evaluated by quantifying resistance forms in rehydration experiments. First, we expect ecosystem functions to show optimal functioning near current climatic conditions, so multifunctionality will decline or collapse under prolonged stress due to sublethal effects on influential species and/or mortality. Second, we predict that most invertebrate species will not withstand dehydration in the active larval or adult stage (weak ‘internal resilience’), so biotic resilience will mostly rely on immigration from source patches. Ecologists have a limited timeframe in which studies on consequences of climate change will be informative/useful to society, so need to seek experimental shortcuts by which generic mechanisms can be brought out. While providing high-quality measures of ecosystem level physiological response and resilience capacity to drought, this project will provide a fresh approach on how to predict the ecosystem consequences of climate change.

Systematics and biogeography of the Slender-legged tree frog (*Osteocephalus taurinus*) species complex in Amazonia

PI: Antoine Fouquet & Diego Ortiz

LEEISA, Cayenne & College of Science and Engineering, Australia

Abstract: The Slender-legged tree frog *Osteocephalus taurinus* species group is endemic to Amazonia and includes a complex array of genetic lineages and cryptic species (hidden diversity). Currently, its taxonomy and phylogenetic relationships remain poorly understood. Given its wide distribution across Amazonia and strong association with primary rain forest, our study system is ideal to test biogeographic hypothesis on the origin of the Amazonian biodiversity, notably the role of large rivers. We will use genomic data, as well as model-based statistical approaches on a large data set of individuals to address this problematic at the scale of the entire region. The results will have a direct impact on the knowledge and conservation of the species by unambiguously defining taxonomic units (at species and population levels) and its geographic distribution, estimate timing of diversification, and identifying evolutionary and demographic processes and past and current centers of diversity. Very few vertebrate groups have been sampled at such a fine scale over the entire Amazonia, therefore our results could represent a good indicator of a more general pattern of diversity and evolutionary history for Amazonian biota. Subsequently, such knowledge will contribute to improve effective conservation and management plans at several geographic scales in the region.

The sense of directions: sensory ecology and navigation in the rainforest.

PI: Andrius Pašukonis, Max Ringler & Maxime Garcia

Harvard University, USA; University of California, USA & ENES, Lyon

Abstract: Detailed knowledge of the sensory ecology of rainforest animals is key for understanding

their interactions with the environment. Most motile animals face the challenge of integrating environmental cues to recognize places and to navigate between them. Animal movement and orientation mechanisms have been studied across spatial scales and taxa, but primarily in animals moving in open space. Thus, very little is known about the cues used by animals to find their way around in cluttered environments such as the rainforest understory. Poison frogs (Dendrobatidae) are a group of common diurnal frogs of the rainforest understory with complex spatial behavior. Recent research has revealed surprising navigational abilities in these small frogs. Despite these advances, as for the most rainforest animals, the cues that they use for orientation remain a complete mystery. In the proposed project we will attempt to fill this gap of knowledge by measuring fine scale environmental variations potentially relevant for the frogs' sensory ecology and relate them to the navigational performance of experimentally translocated frogs, using telemetry. More specifically, we aim at: (1) quantifying fine-scale environmental variations, potentially sensible by terrestrial diurnal frogs; identifying and disentangling (2) the environmental factors that favor frog movement in general from (3) biotic and abiotic cues that influence their spatial orientation. We will use a common and well-studied dendrobatid frog *Allobates femoralis* to address these questions. However, given the ubiquitous challenge of orientation, and widely shared sensory modalities across taxa, our findings should transfer well to other species in similar environments and thereby be of broad interdisciplinary research interest. The results of this project will lay ground for future experimental work, directly manipulating cues used by rainforest amphibians for navigation.

Seasonal variations in diterpenoid composition of *Sandwithia guyanensis* Lanj. and *Sagotia racemose* Baill.

PI: Marc Litaudon
ICSN, Paris

Abstract: *Sandwithia guyanensis* and *Sagotia racemosa* are two endemic species of the family Euphorbiaceae of the Guyana Plateau region. Considered as sister species, they are unique from a taxonomic point of view, belonging to two genera including only 2 species each. Some preliminary investigations showed that these species biosynthesize an array of original secondary metabolites, among which macrocyclic and polycyclic diterpenoids possessing extremely rare carbon skeletons. It is admitted that these two subclasses of metabolites, generally endowed with very interesting biological properties (PKC modulators, P-gp inhibitors, etc), could play a prominent role in the chemical defense of the plants toward attack of herbivorous. In addition, the structure of the molecules and their features are often used as elements to infer taxonomic and phylogenetic relationships between specific plant groups. The basal levels of these metabolites for each organ, tissue or cell type depend on the development stage and they may be modified as a response to biotic and/or abiotic stress. Some qualitative and quantitative variations can also be observed between individuals, seasons or according to external factors. In the present project, we propose to investigate qualitatively and quantitatively the seasonal variation of the two subclasses of secondary metabolites of the two species according to a basic experimental model. The variation of the chemical composition of various samples will be assessed using an innovative analytical approach (named Molecular Networking), which allows the clustering and visualization of secondary metabolites according to their chemical similarity (19). Based on LC/MS/MS analyses, this new methodology has been improved in our laboratory by the ability of carrying out a reliable quantitative analysis of data. If the proof of concept is validated, this methodology could be easily applied to many other comparable studies, thus accelerating fundamental knowledge about the role of these secondary metabolites

VERTIcal stratification and functioning of the funGal and bactErial microbial communities in the canopy of atropical rainforest

PI: Heidy Schimann

EcoFoG, Kourou

Abstract: Leaves are colonized by a huge diversity of microbial species named the phyllosphere microbial communities (hereafter PMCs). Early studies on microorganisms on tropical trees leaves suggested that PMCs have the ability to fix atmospheric nitrogen and may provide nitrogen to the plant. Since then, the significant influence of PMCs on the functioning of tropical forests - pathogen mitigation, plant/insect interactions or seedling survival - have been demonstrated. Some of these microorganisms become saprotrophs after the onset of the senescence of the leaf. A current challenge is to quantify the positive, neutral and negative effects of foliar microorganisms on the physiology of tropical trees, and integrate these findings to assess the impact of PMCs on functioning of forest canopies. The variations in the chemical composition of the tropical trees leaves associated with the strong light and water gradients create a vertical stratification in PMCs structure and composition. However, no quantitative finescale studies exist linking the structure and function of PMCs to within-canopy variation in leaf microclimate and leaf functional traits. In neotropical rainforest, N-fixing trees species play a major role in the N-cycle through their symbiotic interactions with soils diazotrophs, but the distribution of N-fixing bacterial communities in the canopy of tropical forests or the differences of their communities between nodulating and non-nodulating tree species has not been studied so far. Combining functional plant traits, accurate microenvironmental data and metabarcoding, the VERTIGE project aims at exploring the PMCs in tropical rainforest trees, particularly looking at the influence of the light and humidity gradients associated to foliar characteristics shape PMCs. We will test the hypothesis that N-fixing epiphytic bacterial communities are more diverse on top of the canopy where light is non limiting than in the understorey and whether they are richer in non-nodulating tree species. On the opposite, saprotrophic fungal endophytic communities might be more diverse in the understorey. This innovative and original approach will give new insights into the understanding of how microbial diversity is related to host function, both considering PMCs as a potential functional trait or how they mediate other plant traits.