TITRE du projet	**Evaluating the impact of diet, microbiota and environmental factors on vectorial capacity of vectors of water-borne zoonotic diseases.**
Acronyme	MICROVECT
Mots Clefs y compris modèles biologiques d'études	vectorial capacity / competence, microbiota, diet, water, *Galba truncatula, Bulinus truncatus, Aedes albopictus, Culex pipiens,* fasciolosis, schistosomiasis, arbovirus.
Objectifs du projet	The mechanisms and factors promoting the (re-)emergence of most vector-borne diseases are still poorly understood making any predictions on their establishment and spread particularly challenging. Obviously, the presence of competent vectors is mandatory for a given pathogen to establish and maintain locally. Nonetheless, the ability of locally established vectors to ensure an endemic/or epidemic pathogen transmission (i.e. their "vectorial capacity") greatly depends on several factors (density, longevity, fecundity, behavior) modulating the contact between the vertebrate host and the vector and thus the ability of the vector to potentially amplify the pathogens, the so-called **vector competence**. Vectorial capacity is also largely influenced by numerous biotic and abiotic factors including pollution, temperature, land use and nutrition, because they affect vector population densities and vector-host contact. These factors also alter the vectorial capacity by modifying the vector competence through metabolic and/or immune intrinsic changes. Predicting the emergence (and controlling transmission hotspots) of some vector-borne pathogens thus requires identifying (and controlling) those factors with the strongest influence on vector global competence. **The present project aims at deciphering the links between environment, environmental microbial communities, microbiota, diet and vector competence of intermediate hosts and vectors of zoonotic water-borne diseases.** More specifically, we will assess the direct and indirect effects of diet/food resources and environmental microbial communities/microbiota on vector competence of four gastropod and arthropod vectors locally established in freshwater ecosystems of France Mediterranean region
Durée du projet	2021-2025
Equipe porteuse du projet	-**Partner 1:** UMR 5244, Interactions Hôtes Pathogènes Environnements (IHPE), UM, CNRS, IFREMER, UPVD (Benjamin Gourbal, Olivier Rey, Juliette Langand, Eve Toulza, Mathilde Jaquet) Porteur : Benjamin Gourbal benjamin.gourbal@univ-perp.fr Tel : 04 68 66 20 50

Partenaires	-**Partner 2**: UMR MIVEGEC, Département PEV (Perturbations, évolution et virulence), Montpellier (Elodie Chapuis, Dominique Faugère, Sylvie Hurtrez-Bousses) -**Partner 3**: UMR MIVEGEC, Département ESV (Evolution des Systèmes Vectoriels), Montpellier (Thierry Lefèvre, Christophe Paupy, Davy Jiolle, Philippe Bousses) -**Partner 4**: UMR CEFE, équipe Génétique et Ecologie Evolutive, Montpellier (Philippe Jarne, Patrice David) -**Partner 5**: UMR SETE, Moulis (Simon Blanchet, Murielle Richard) -**Partner 6:** Institut de recherche pour la conservation des zones humides méditerranéennes, Tour du Valat, le Sambuc, Arles (Marion Vittecoq)

➢ **State of the art and objectives of the project**

Freshwater constitute vital resources for most living organisms including Humans. Paradoxically, freshwater ecosystems are amongst the most degraded ecosystems by human activities due to pollution, fragmentation, overexploitation and the introduction of non-native species (*1-3*). Such deep environmental modifications have fostered the transmission locally or in new transmission area (emergence) of several water-borne diseases and vector-borne diseases induced by parasites with complex cycles transmitted by arthropods and gastropods. This is the case for fasciolosis, caused by the liver fluke *Fasciola hepatica*, an infectious disease transmitted by several vectors including the snail *Galba truncatula* and causing serious damages in livestock and occasionally in Humans across Europe (*4, 5*). Moreover, due to global warming and the increasing dispersal opportunities induced by anthropic international trades, several organisms that act as vectors for tropical vector-borne diseases are shifting their geographical range Northward and pave the way for future emergences of several infectious diseases into more temperate regions. For instance, *Aedes albopictus* is established in Southern Europe since the 2000's and is now reaching Northern Europe (*6, 7*). This mosquito is a vector of several infectious diseases including chikungunya and dengue (*8*). So far however and fortunately, very limited endemic infections occurred for most of these arthropod-borne infectious diseases (*6*). Besides invasive vector species, common native European mosquitoes (mainly from the genus *Culex*) are increasingly involved in the transmission of emerging and re-emerging zoonotic viruses, including the Usutu and West Nile viruses, two viruses of medical and veterinary interest (*9, 10*). Similarly, populations of *Bulinus truncatus* snails are established in Spain, Italy, Greece, Portugal and in most Mediterranean Islands and raise the risk for future establishment of bilharziasis, the most prevalent parasitic disease after malaria in Africa. In fact, bilharziasis emerged during Summer 2013 in Corsica with recurrent outbreak the following years, where hundreds of endemic infections have been reported so far (*11, 12*).

The mechanisms and factors promoting the (re-)emergence of most vector-borne diseases are still poorly understood making any predictions on their establishment and spread particularly challenging. Obviously, the presence of competent vectors is mandatory for a given pathogen to establish and maintain locally. Nonetheless, the ability of locally established vectors to ensure an endemic/or epidemic pathogen transmission (i.e. their "vectorial capacity") greatly depends on several factors (density, longevity, fecundity, behavior) modulating the contact between the vertebrate host and the vector and thus the ability of the vector to potentially amplify the pathogens, the so-called **vector competence** (*13*). Vectorial capacity is also largely influenced by numerous biotic and abiotic factors including pollution, temperature, land use and nutrition, because they affect vector population densities and vector-host contact (*13*). These factors also alter the vectorial capacity by modifying the vector competence through metabolic and/or immune intrinsic changes (*14*). Predicting the emergence (and controlling transmission hotspots) of some vector-borne pathogens thus requires identifying (and controlling) those factors with the strongest influence on vector global competence.

The microbiota (community of microorganisms, including bacteria, viruses and fungi) and the diet (food resources) of an organism are likely to be a central piece in the triptych composed of hosts/vectors, pathogens and environment. First, the microorganisms' assemblage at the ecosystem level is largely influenced by the local environmental conditions. Second, a fraction of the microbial communities of the environment is intimately linked to vectors, the so-called microbiota and such microbiota is strongly influenced by the diet of the organism considered. Indeed it has been demonstrated, mainly for arthropods, that quantitative and qualitative variations of diet resources could affected the relative abundance of the microbiota communities and microbiota composition (*15-17*), suggesting that microbiota assembly is primarily diet-driven.

Then the microbiota affects several vital functions in hosts including metabolism (*18, 19*) and immunity (*20, 21*). For instance, the microbiota can produce reactive oxygen species (ROS) against pathogens (e.g. Enterobacter in *Anopheles gambiae*; (*22*), elicit a basal immune activity (e.g. mid-gut microbiota in *Aedes* sp. (*23*) or induce immune genes (e.g. *Wolbachia* in *Anopheles* sp.; (*24*). Alternatively, the microbiota can "shape" the immune system of its host (*25*). In molluscs, the unicellular Eukaryota *Capsaspora owczarzaki* can confer a protection to its host *Biomphalaria glabrata* against the trematode *Schistosoma mansoni* (*26*). Third, the microbiota is influenced by both the physiological status of hosts and environmental conditions. For instance, the gut microbiota is partly influenced by the age and stress level of its host but more particularly by the diet (as stated above), which directly

depends on the external environment. It has been demonstrated in insects, that diet supplementation (nectar rich in micronutrients) improves gut microbial diversity and abundance, resulting in a strong line of defence against biotic stressors and improving individual lifespan (*27*).

Thus the microbiota is at the interplay between the environment (microbiome: microbial communities of the environment) and the physiological status of vectors / hosts including metabolism and immunity and hence constitutes a key component of vectorial capacity or competence (Fig. 1). Moreover, microbiota composition and diversity is potentially controlled by quantity and quality of the diet and food resources available in the environment, thus variation in diet resources due to contrasted ecosystems or to seasonal variation are expected to have significant impacts on individual microbiota composition and therefore on their vectorial capacity or competence (Fig. 1).

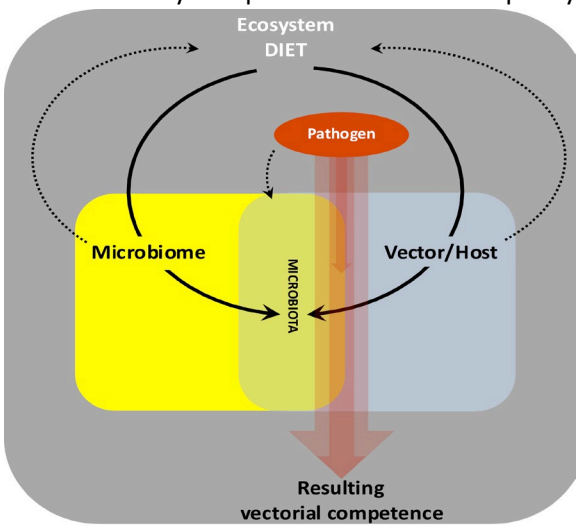

Figure 1: Schematic representation of environmental and intrinsic factors influencing vector competence. Black arrows indicate direct relationship, dashed arrows reciprocal interactions. Ecosystem and diet, both influence the microbial communities of the environment and the vector microbiota. Diet influence vector biological, physiological, metabolic and immune traits that shape the microbiota. The conjunction of all these factors defined the vector competence, and thus the higher or lower potential for a given vector to transmit pathogen (red arrows).

In mosquitoes, gut bacterial microbiota strongly influence vector competence and can be determined by both the aquatic breeding habitats which provide resources for larval development and the adult dietary regime (*28-30*). Availability of food resources have been demonstrated to determines their survivorship, growth, longevity, body size, flight capacity, survival rate, fecundity and host-seeking behaviour, which may determine the vectorial capacity of mosquito populations (*31*). In Culex mosquitoes for instance, individuals originating from high-nutrient habitats were associated with members of the Clostridiales order, while those from low-nutrient habitats were instead associated with Burkholderiales order members (*32*). The source of blood meal may also influence the composition of the microbiota in mosquito midguts. In particular, members of the genera *Leucobacter*, *Chryseobacterium*, *Elizabethkingia*, and *Serratia* were characteristic of *Ae. aegypti* adults fed on chicken, rabbit, and human blood, respectively (*33*). Besides feeding on blood, mosquito females readily feed on natural sources of plant sugars in the field which can host a great diversity and abundance of bacterial species (*34*). Natural sugar sources for adult mosquitoes, i.e., specific plants providing high metabolic energy, might affect mosquito biological traits and their capacity to transmit pathogens. Whether these sugar meals impact the dynamic of mosquito microbiota, and hence their competence for pathogens, remains unknown. Moreover, most of the studies conducted on mosquito diet and vectorial capacity have been carried out under laboratory conditions, highlighting the need for studies on mosquitoes under field conditions. Recent studies also indicate that mosquito gut-associated microbiota can impact vector density, biting rate, survival, vector competence, and the pathogen extrinsic incubation period, and therefore ultimately modulate vectorial capacity (*35*). However, less attention has been paid to how microbiota affect phenotypes that impact such vectorial capacity (*35*), and there are still many knowledge gaps regarding mosquito-microbe-dietary regime interactions that need to be addressed.

Concerning gastropods, everything or almost everything has to be done. Less is know for snails concerning variation in the availability and quality of diet and their influences on biological traits like gut microbiota community composition or diversity, metabolism, immunity and as a consequence how this affects their vectorial capacity or competence to different pathogens (*21, 36, 37*).

The present project aims at deciphering the links between environment, environmental microbial communities, microbiota, diet and vector competence of intermediate hosts and vectors of zoonotic water-borne diseases (Fig. 1). More specifically, we will assess the direct and indirect effects of diet/food resources and environmental microbial communities/microbiota on vector competence of four gastropod and arthropod vectors locally established in freshwater ecosystems of Occitanie region. Each biological model, and the parasite they

transmit, shows different degrees of endemicity in Occitanie region. 1. *Galba truncatula* is locally established and transmit, among others, *Fasciola hepatica*, a trematode responsible for the liver fluke in Humans and animals. 2. The invasive mosquito *Aedes albopictus* and the native *Culex pipiens* are also well-established locally. These vectors of several pathogens of public health relevance are considered as a threat for future outbreaks in Occitanie (*A. albopictus*: chikungunya and dengue viruses, *Culex pipiens*: West Nile and Usutu viruses). 3. *Bulinus truncatus* is not locally established (although reported in the mid-20th century near Perpignan), but occurs in several neighbouring regions (e.g. Spain, portugal, Corsica). *Bulinus truncatus* transmits *Schistosoma haematobium*, a trematode responsible for the urogenital form of bilharziasis. This tropical neglected disease is in the way of endemicization in Corsica. Due to the ambitiousness of the project, we propose to focus the study on vector competence rather on the global vectorial capacity of these four host-vector systems using an integrative approach from the molecular level towards the ecosystem. The project gathers scientists with complementary theoretical and analytical skills that encompass all these biological levels. Although challenging, such multidisciplinary and integrative approaches are now possible thanks to technological and analytical advances. All the approaches conducted in MICROVECT will be an important step toward implementing system biology and mathematical models for predicting vector competence in biological systems.

This integrative project will help in a near future to i) identify the relative importance of the various biotic and abiotic characteristics of the ecosystem in the competence of transmission and vectorial capacities, ii) help in implementing models of transmission dynamics and vector competence of gastropods and arthropods according to the key environmental parameters characterised. The models will serve as predictors helping in quantifying changes in environmental factors impacting the vector competence and capacity, iii) help in predicting hotspots of transmission or evaluate the epidemiological risk in specific natural ecosystems, iv) and finally, make possible links with the RIVOC project "Action publique, risque, surveillances et contrôle durable" that will be launch next year.

MICROVECT is structured into four work-packages (WP), and the consortium includes 5 Partners in the "Occitanie region" that are leaders in their respective skills with a recognized international expertize either on specific technical challenge or research fields on arthropods and gastropods. The project has been structured to ensure efficient interactions among researchers and sharing of their respective technical and theoretical backgrounds, biological material and laboratory infrastructures and stuffs. One PhD student (to be recruited, co-direction by Partner 1 and Partner 3) sharing time between partner laboratories will help in the proficiency of the project.

- **A complementary set of Work-Packages:**

WP1: Collaborative review (all Partners involved + PhD student recruited)

This first WP aims at producing a review on the influence of environmental biotic and abiotic factors on vectorial capacity and vector competence of gastropods and arthropods. This review will be a federative work between all Partners, and will be initiated from the beginning of the project. This WP will have a first virtue of a collaborative effort towards a focused goal for the whole consortium. This review will benefit from the wide disciplinary competence of the consortium that will permit to produce a comprehensive synthesis on vectorial capacity and competence in a wide context from molecules to populations and ecosystems. The aim of this review is to critically revise the current knowledge on the effects that both biotic and abiotic factors have on vectorial capacity, identifying the critical knowledge gaps and proposing future research lines. This type of review is currently needed and will highlight opportunities for novel vector or intermediate host control strategies. Moreover, this review would be also a very interesting exercise for the future PhD student in order to start his/her thesis bibliography and having a nice basis for the introduction chapter of his/her thesis.

This collaborative review and the scientific papers to be published in the framework of the MICROVECT project will be published in general "rank A" journals with large audience. All the Partners of the present project will support open science by deposit all the scientific productions related to the project on open read archives and especially HAL (https://hal.archives-ouvertes.fr/).

WP2: Identifying environmental parameters (diet, microbiota) that shape the plasticity of vector competence in natural ecosystems: (all Partners involved + PhD student recruited)

The availability of food resources and its link with microbiota composition is one of the key factors influencing individual biological traits, although their roles in arthropods and gastropods pathogen transmission remain unclear. Most of the studies done so far have been carried out under laboratory conditions and mainly for arthropods, highlighting the need for studies investigating the link between feeding behaviour (diet) and microbiota under field conditions. Thus, the aim of the WP2 is to characterize the vector competence of the four vector models considered, in a spatial and temporal comparative analysis of contrasted ecosystems (Annex 1) in which diet food resources and environmental microbial communities varied qualitatively and quantitatively.

This WP2 will allow us i) assessing to which extent the vector competence of each vector is diverse among the ecosystems, ii) assessing whether the vector competence of distinct vectors was similar when confronted to similar environmental conditions; iii) identify the influence of food resources and microbiota with diversity in vector competence in the field and iv) characterise microbiota and diet associated with high or low vector competence in the field.

Because the four vector species do not live in sympatry, specific sites were selected for each species (Annex 1) with the idea of maximizing sampling areas in common among vectors, resulting in 5 areas and 2 sites per area (Annex 1). Repeated temporal sampling (four times a year) will be performed at each field site to assess the temporal dynamics of vector competence for each species. The sampled individuals will be brought to the laboratory and their competence will be evaluated by measuring the prevalence and intensity of pathogens. Prevalence will be estimated by PCR diagnostic using specific primers designed to discriminate trematode infections in snails and viral infections in mosquitoes. For snails the ability of transmission (intensity) will be quantified by measuring the cercarial shedding. In mosquitoes, quantitative PCR (Q-PCR) will be used to estimate viral infection and viral load. All these PCR, Q-PCR en experimental parasite quantification are yet developed and mastered by Partners 1, 2, 3 and 4 on their respective biological models. Due to sometime low prevalence in the field, laboratory experimental infections following by quantification of prevalence and intensity of infection will be also tested on individuals recovered from the various field ecosystems to evaluate experimentally their vector competence. These experimental infections will be useful to correlate variations in environmental parameters (diet, microbiota) found in natural ecosystems with high or low vector competence. All the breeding and P2 experimental facilities to manipulate vectors and their respective pathogens are available in Partner laboratories.

Concomitantly in each field site, the individual microbiota composition and food resources (diet) will be analysed. The environmental microbial communities and vector microbiota community composition and diversity will be determined using Miseq 16S barcoding. The environmental microbial communities at the ecosystem level will be characterised from water, sediment and plant nectar samples collected at each site and at regular times in order to obtain an exhaustive view of locally established microbial communities. All samples will be individually frozen in liquid nitrogen, DNA will be extracted, quantify and Individual 16S rDNA amplicon libraries will be generated targeting the variable V3-V4 loops of 16s rDNA. Aside from the vector competence, individual measures of diet will be also performed. Indeed, the originality of the project lies in the fact that we correlate the vector competence to microbiota and to individual diets. Gastropods feed mostly on unicellular and filamentous algae, aquatic plants and dead organic matter. Concerning mosquitoes we will focus our analysis on adult stage diets and more precisely on plant floral and extra-floral nectar ingested by both adult sexes as nutritional sources in the field (*38*). For both gastropods and arthropods, we will test how variations in the availability and quality of diet affect their vector competence to trematode and viral pathogens, and we will determine if snails or mosquitoes transmitting pathogens were associated with specific microbiota communities or have ingested a specific diet (*39, 40*). DNA metabarcoding will be use to evaluate the plant component of vector diets using a Chloroplast DNA marker the trnL-P6 that has proven to be very informative (*41*). We will used DNA extracted from individuals sampled in the field, but also environmental DNA (water/sediment/plant nectar samples) and fecal samples recovered from field individuals maintained few hours in laboratory before being frozen in liquid nitrogen and processed for DNA extraction as stated above. For microbiota and diet characterization, sequencing will be performed on the Illumina MiSeq using the facilities of Epigenomic platform and Bio-Environment platform housed by Partner 1 laboratory and the University of Perpignan respectively. The FROGS pipeline implemented on a galaxy instance of Partner 1 will be used for data processing.

Moreover, to go further in the description of the environments in which our species of host and vector evolve, the abiotic local environmental conditions will be fully characterized at each sampling site and time. This includes

numerous physico-chemical parameters based on water samples and *in situ* analyses using the ODEON portable unit multi-parametric device (temperature, % O2 saturation, turbidity, conductivity, salinity, pH, redox balance). Finally, as host' genetics is thought to influence positively or negatively the microbiota structure and diversity, we propose to control individual and population genetic profiles as a potential confounding factor. We will assess the neutral genetic diversity and genetic structure of vector populations based on microsatellites. Parameters of population genetics will serve to estimate intra and inter-population diversity. We will compare the genetic diversity of vectors sampled in each field sites and make potential link with their microbiota and their vector competence. This will give solid information on intra-, inter-specific microbiota stability based on host genetic differentiation in field-contrasted environments. Individual DNA samples extracted for microbiota analysis (see above) will be also used here for the genetic approach. Protocols for *G. truncatula* and *B. truncatus* genotyping will be conducted according to procedure developed by Partners 1, 2, 4 laboratories. For *A. albopictus* and *C. pipiens* spatial and temporal genetic variation have been yet characterized (Partner 3) and thus DNA samples will be keep for additional genotyping if necessary. Microsatellite PCR products will be analyzed using the platform GenSeq (LabexCemeb) facilities.

WP3: Assessing the links between diet, microbiota and vector competence under controlled experiments (all Partners involved + PhD student)

Based on WP1 and WP2 results, we will have a good overview of the microbiota diversity at the inter- and intra-specific levels and in contrasted natural environments submitted to different diet (metabarcoding of food intake) and their influences on vector competence plasticity. In the WP3 we will quantify experimentally the influence of diet and microbiota on vector competence based on controlled laboratory and mesocosm experiments.

We propose to i) manipulate the microbiota (antibiotic treatments and/or microbiota reciprocal transfers), in order to change the microbiota composition and understand the key role played by microbiota on vector competence; ii) manipulate the food resources a diet of organisms to infer the impact on microbiota and vector competence; iii) study the dynamics of the microbiota following parasite infestation. By modifying mesocosm parameters (thermal regimes, net primary productivity, food availability and quality) we expected to change diet consumption and microbiota community composition leading to strong impact on vector competence.

To assess the causal links between diet, microbiota, and vector competence the following experiments will be conducted:

- Laboratory controlled experiments: **(Partners 1, 3 and 4 breeding facilities)**: In gastropods, microbiota alteration by antibiotic treatments will be conducted on adult snails. We will use 7 antibiotics from six classes with the goal of differentially altering the microbiota (impacting specific microorganism families or genders) and assessing their impact on vector competence following experimental infection (for prevalene and intensity measures see WP2). Microbiota before and after antibiotic treatments will be characterised (see WP2 for details on microbiota sequencing). To evaluate possible links between specific microbiota communities and vector competence, we propose to carry out also reciprocal transfers of microbiota between individuals with high or low vector competence identified in WP2. The efficiency of the transfer will be verified by 16S metabarcoding and the quality of the colonisation will be analysed by comparison with vector populations identified on WP2 field studies. In mosquitoes, antibiotic treatments are not feasible, due to endosymbiotic wolbachia suppression and consequently high fitness cost. Thus an alternative approach will be conducted. Before the experimental infection, mosquitoes will receive nectar form wild plant species identified as part of WP1. Following pathogen incubation 1) vector competence will be quantified using conventional parameters (infection rates, dissemination rates and viral load in saliva), 2) among plant differences in mosquito microbiota will be assessed. Experimental approach will be conducted in controlled BSL3 insectary (Vectopôle. Centre IRD Partner 3) for mosquitoes exposed to CHIK and West-Nile Virus.
- Manipulative experiments on mesocosms **(Partner 5 infrastructure facilities)**: Influence of diet on microbiota and vector competence. The mesocosm experiment will be conducted exclusively on *Galba truncatula* (see Annex 1). Indeed, *Bulinus* could not be used due to the risk of spreading in the Lez River.

This mesocosm experiment will be developed in a unique facility (the Aquatic Metatron) in the SETE partner. This facility encompasses 144 large tanks (2000L) in which realistic lentic ecosystems can be created and in which water

temperature can be adjusted and controlled for each tank independently (https://sete-moulis-cnrs.fr/en/services/experimental-platforms/aquatic-metatron). We will assemble realistic contrasted ecosystems by seedling each tank with dead organic matter (leaf litter), primary producers (phytoplankton and macro-algae) and secondary consumers (zooplankton). Following alteration of microbiota by antibiotic treatments in laboratory, vectors will be reared and acclimated to two different mesocosm environments varying in terms of thermal regimes, of net primary productivity and food availability and quality. Each treatment will be replicated in 9 independent tanks. The platform allows continuous recording of several parameters such as temperature, oxygen concentration, pH, conductivity and chlorophyll a concentration. Elevated concentrations of chlorophyll *a* can reflect an increase in nutrient loads, with high numbers of phytoplankton and free-floating macro-algae. By manipulating habitat characteristics, we aim at altering the environmental microbial community's composition, and diet consumption allowing testing for their impact on vector microbiota communities and vector competence. The experiment will run for 12 months to allow the setting-up of dissimilarity. Sampling will be conducted each two months and the vector competence will be evaluated by measuring after experimental infections, the prevalence and intensity as described in WP2. The environmental microbial communities, the vector microbiota community composition and the diet composition and diversity will be determined using Miseq metabarcoding as described in WP2.

WP4: Deciphering the molecular support of vector competence: a focus on immunometabolism (Partners 1 and 3 + PhD student recruited)

Vector competence is linked to the level of susceptibility to pathogens and thus to the snail and mosquito immune capacities. Interestingly, it has been demonstrated that metabolism could be linked to immune defence capacities (*42, 43*). Moreover, metabolism is strongly impacted by environmental factors and microbiota. It has been demonstrated that microbiota i) affects coordination of general metabolism and allocation of resources scheme (*18, 19*); ii) exerts important metabolic functions, iii) regulates the inflammatory response by stimulating the immune system and iv) has effects on epithelial barrier and immune internal defence system (*20*). Thus, due to environmental factors, and diet, the microbiota could be affected and may change the metabolism of vectors, resulting in a modification of their immune ability, and consequently increase or reduce their vector competence. Here we will use the animals subjected to different environmental conditions of WP2 and WP3 and analyse their immune capacities and their global metabolism. This approach will be useful to decipher the molecular processes or pathways supporting vector competence and to identify biomarkers of high or low vector competence.

First, metabolomic changes will be investigated for whole animals and hemo-lymphatic compartment using a non-targeted approach without *a priori* information or targeted approaches, using selected reaction monitoring (SRM) of 100 metabolites (oxidative phosphorylation, glycolysis, glutamine metabolism, TCA cycle, amino-acids, pentose phosphate pathway, etc …) able to reveal metabolic differences between the experimental groups. Metabolic changes will be investigated in relation to the nature (qualitative changes) and scale (quantitative changes) in metabolites for infected and non-infected animals. Identified and annotated metabolites will then be assigned to metabolic pathways in order to highlight significantly impacted metabolic pathways. All the metabolomic approaches will be conducted using MAMMA platform (BioCampus Montpellier) facilities.

Second, innate immune capacities of animals will be also investigated. Using genomic and transcriptomic data (annotated genome, RNAseq data), snails and mosquito immunome (list of key immune genes, i.e., RNAi pathway for mosquitoes) will be constituted. The differential expression of these candidate immune genes will be validated by quantitative real time PCR (Q-RT-PCR). This will be done on whole animals when possible using individuals sampled in WP2 and WP3. This approach will help us to confirm the differential expression of candidate genes and provide insight into immune mechanism associated with vector competence. Q-PCR will be conducted on LightCycler® 480 Real-Time PCR System available at Bio-Environment platform University of Perpignan and using the "QPCR haut debit" platform of labex CEMEB, University of Montpellier.

Third, constitutive hemocyte subpopulation profiles and counting will be monitored by Flow Cytometry analysis (FCM). Indeed, changes in number, shape or internal structure of the hemocytes are key parameters of immune capacities and thus of potential competence to transmit pathogens. All these cellular approaches will be performed using a Montpellier RIO Imaging Platform (MRI), BioCampus Montpellier facilities. Phagocytic and lytic capacities of hemocytes will be tested *in vitro* in primary cell culture using cell culture facilities of Partner 1.

➢ **Estimated Budget requested: 170 k€**

WP1 (6 K€)	**Publication fees:** Open access journal with APC: 6K€
WP2 (75.5K€)	**Field trips:** (Occitanie region, Camargue, Corsica and Spain): 20 K€
	Equipment: ODEON multi parametric water analysis apparatus: 5 k€ Network-attached storage (NAS) 20 To: 1.5 k€.
	Sub-contracting: Environmental genomic platform (microsatellite): 6 k€ BioEnvironment platforms (MiSeq, 1000 samples): 20 k€ Oligo, Sanger sequencing (Eurogentec): 3 k€
	Material, consumables: Molecular biology (kits DNA/RNA ext, PCR, q-PCR): 10 k€ Bench material (micro-pipette, plastic stuff, eppendorf): 5 k€ Animal care maintenance and breeding: 5 k€.
WP3 (40.5K€)	**Mesocosm experiment**: 18 tanks renting (12-months), fluid, probes consumption: 8.5 K€
	Laboratory approaches: Biochemistry products, antibiotics: 2 k€ Bench material, plastic stuff, eppendorf: 3 k€ Animal maintenance experimental stuff: snail, parasite: 2 k€ Experimental approach in Vectopôle: 12 k€ Molecular biology (kits DNA/RNA ext, PCR, q-PCR): 3 k€
	Sub-contracting: BioEnvironment platforms (MiSeq 500 samples): 10 k€
WP4 (27K€)	**Material, consumables:** Molecular biology (kits DNA/RNA ext, PCR, q-PCR): 4 k€ Cellular biology (viability assays, media, culture): 4 k€ Bench material (plastic culture stuff): 2 k€
	Subcontracting: FCM, FACS, confocal (plastic stuff, bench material, consumable: 6 k€ Microscopy (fixative, solution, staining, medium): 3 k€ MAMMA plateform (metabo chemical, apparatus renting time): 8 k€
General costs (6K€)	**Administrative and operating expenses:** Consortium meeting, material transfer: 3 k€ Attendance to national/international conferences: 3 k€
Staff expenses (15K€)	Master's gratification (one M1-M2/year, 3 to 6 months at 600€/month): 6 k€ Technician for mesocosm experiment (6 month): 9 k€
TOTAL: 170 K€	

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ANNEX 1:

		Mollusks		Arthropods	
Tasks	**Objectives**	**Bulinus**	**Galba**	**Aedes**	**Culex**
WP1: Collaborative review	Collaborative review on environmental biotic and abiotic factors influencing the vectorial capacity and competence of mollusks and athropods				
WP2: Vectorial competence and characteristation of the environment in natural ecosystems phenology water quality Environmental metabacoding Vector/host metabarcoding (microbiota/diet) genetic background vectorial competence	obj1: Temporal dynamics	Corsica	Tour du Valat	Tour du Valat Montpellier (Botanic Garden, Zoo, recreational city parks)	Tour du Valat Montpellier (Botanic Garden, Zoo, recreational city parks)
	obj2: Spatial level	Corsica Spain	Tour du Valat Coulazou/Prades-le-Lez (34) Pla d'Avall, Nohèdes (66)	Tour du Valat Montpellier (Botanic Garden, Zoo, recreational city parks) Pla d'Avall, Nohèdes (66)	Tour du Valat Montpellier (Botanic Garden, Zoo, recreational city parks) Pla d'Avall, Nohèdes (66)
WP3: Assessing the links between diet, microbiota and vectorial competence under controled experiments metabarcoding (vectors' microbiota and diet) genetic background vectorial competence	obj1: Mesocosm (SETE, Moulis)	not done	NPP, temperature modification, qualitative and quatitative diet variation, microbiota variation	not done	not done
	obj2: Laboratory experiment	antibiotic treatment, diet composition, common garden, phenotypic plasticity	antibiotic treatment, diet composition, common garden, phenotypic plasticity	antibiotic treatment, diet composition, common garden, phenotypic plasticity	antibiotic treatment, diet composition, common garden, phenotypic plasticity
WP4: Deciphering the molecular support of vectorial competence Biological material from WP1 and WP2	obj1: Immune capacities	immune gene expression hemocyte profil flow cytometry phagocytic lytic capacity	immune gene expression hemocyte profil flow cytometry phagocytic lytic capacity	immune gene expression hemocyte profil flow cytometry phagocytic lytic capacity	immune gene expression hemocyte profil flow cytometry phagocytic lytic capacity
	obj2: Global metabolism	non-targeted metabolism targeted metabolism seahorse approach	non-targeted metabolism targeted metabolism seahorse approach	non-targeted metabolism targeted metabolism seahorse approach	non-targeted metabolism targeted metabolism seahorse approach