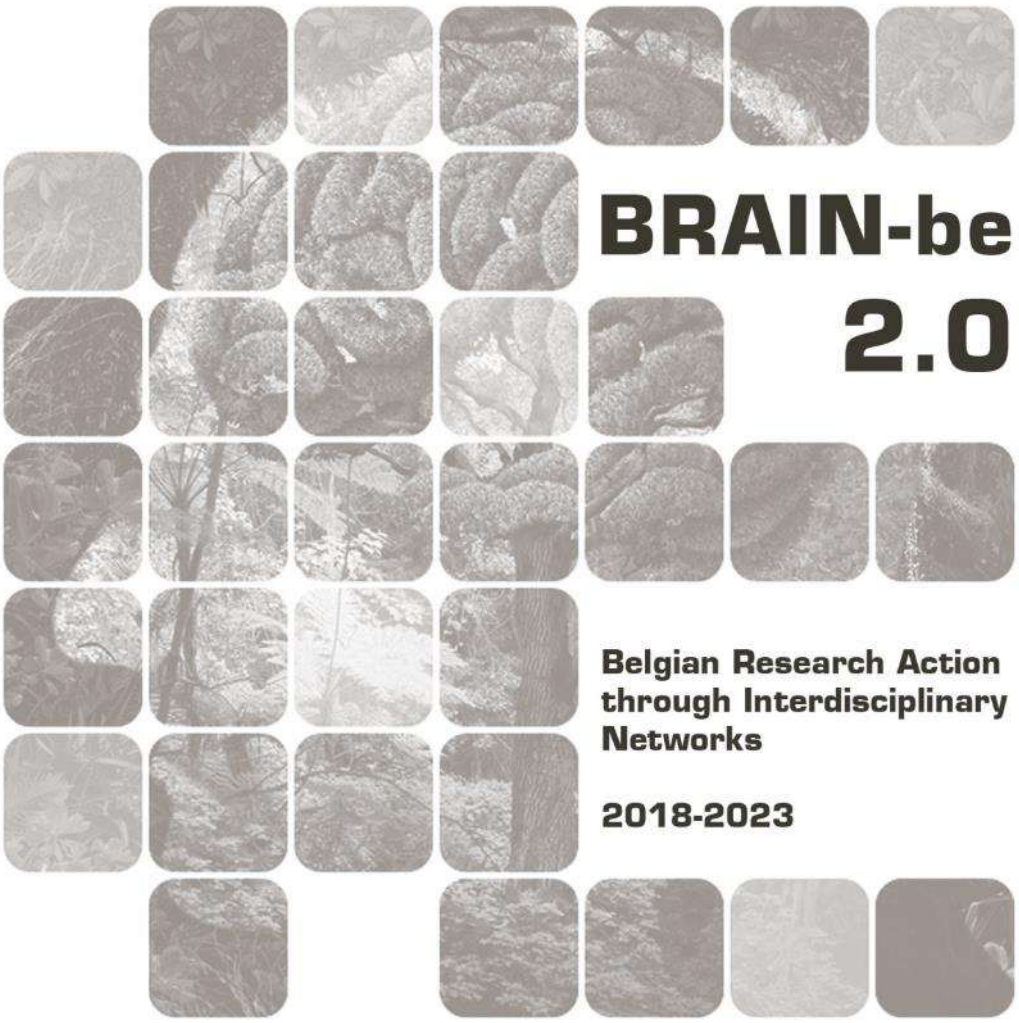

MicroResist

The influence of snail host microbiome in trematode parasite resistance

Ruben Schols (RMCA & KUL) – Ellen Decaestecker (KUL) – Tine Huyse (RMCA)

Pillar 1: Challenges and knowledge of the living and non-living world

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NETWORK PROJECT

MicroResist
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FINAL REPORT

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ABSTRACT

Context

Trematodiases, or snail-borne diseases, are caused by trematode infections and have global implications. These diseases affect approximately 250 million people, over a billion livestock, and countless wild animals, causing significant health, economic, and conservation challenges. Environmental change, global transport, and climate shifts exacerbate the spread of freshwater snails and their parasites, altering transmission dynamics and expanding the diseases' geographic range. Existing control efforts, focused largely on drug treatments, have achieved limited success due to anthropogenic influences, parasite hybridization, and the role of animal reservoirs. Research suggests that targeting the microbiome of freshwater snails could provide an innovative approach to enhancing resistance to trematode infections.

Objectives

The MicroResist project aimed to investigate the role of the bacterial aspect of the microbiome in snail resistance to trematode infections through four key objectives. First, it explored how the snail microbiome changes during the maturation of *Schistosoma* infections, considering co-infections, developmental stages, and parasite species. Second, it validated and improved a method for creating germ-free snails and developed a robust microbiome transplant protocol. Third, the project examined the link between the microbiome and resistance in a globally invasive snail through reciprocal transplant experiments. Lastly, it focused on invasive snails and their ecological impacts within man-made lakes.

Conclusions

The project revealed that parasite exposure shapes bacterial communities in freshwater snails but found no direct evidence of microbiome-induced resistance to trematode infections. Nevertheless, these findings provide essential tools and insights for future research, including microbiome-based interventions to enhance resistant snail populations. The work also highlighted that the enigmatic common hippopotamus experiences increased parasite exposure due to invasive snails, potentially complicating conservation efforts. Moreover, it revealed the urgent need for strengthened biosecurity measures, systematic monitoring to prevent invasive snail spread, and international collaboration to address the ecological and health challenges posed by trematodiases. By advancing our understanding of microbiome-parasite interactions, the MicroResist project contributes to the broader goals of biodiversity conservation, public health, and sustainable management of parasitic diseases.

Keywords

Schistosomiasis, microbiome, parasite resistance, museum collections, transplant experiments

1. INTRODUCTION

Freshwater snails

The common name 'snails' refers to the prosobranch and pulmonate representatives of the phylum Mollusca: class Gastropoda (Strong *et al.*, 2008). Prosobranchs are generally characterized by separate sexes, and thus, sexual reproduction; an operculum, a hard plate capable of closing the aperture or shell opening; gills for respiration; and specific habitat requirements. In contrast, pulmonates, which are hermaphroditic and can therefore self-fertilize, lack an operculum, respirate through a rudimentary lung, and have broader environmental tolerances (Strong *et al.*, 2008). Both groups, along with bivalves, are highly diverse and have colonized almost the entire world (Strong *et al.*, 2008). Over 7,000 freshwater mollusc species have been described with an estimated three to eleven thousand still awaiting discovery (Lydeard *et al.*, 2004).

Nevertheless, freshwater snails are one of the most imperilled groups in the aquatic ecosystem, with a third of the species listed as threatened and another third as data deficient (Lydeard *et al.*, 2004; Allen *et al.*, 2011; Böhm *et al.*, 2021). Almost half of the 693 recorded animal extinctions are molluscs (Lydeard *et al.*, 2004). Unsurprisingly, anthropogenic pressures lie at the source through artificial lake construction, water pollution, invasive species, and climate change (Allen *et al.*, 2011). Losing this diversity could have far-reaching consequences as snails are part of complex ecological interactions in the aquatic ecosystem through for example cascading effects of epiphyton grazing (Brönmark, 1989). Additionally, freshwater snails have a major economic impact due to their role as intermediate hosts of trematode parasites, which cause disease in human and animal populations (Allen *et al.*, 2011; Toledo and Fried, 2014).

Trematodes

Trematodes belong to one of the largest clade of obligate parasites, the Neodermata (Phylum: Platyhelminthes), together with cestodes and monogeneans (Littlewood *et al.*, 1999). Trematodes are obligate endoparasites with a complex multi-host lifecycle, usually involving a snail first intermediate host and a vertebrate definitive host (**Figure 1**) (Toledo and Fried, 2014).

The intricate snail-trematode interaction already starts before the intermediate host and parasite come into contact. Chemical cues released by snails can critically shape miracidial finding behavior and thereby transmission success (Magalhães *et al.*, 1997; Hertel *et al.*, 2006). Next, the schistosome must avoid destruction by the snail's immune system to successfully establish an infection (Núñez *et al.*, 1994; De Jong-Brink *et al.*, 2001). This arms-race starts the moment the parasite and snail interact when a miracidium penetrates the head-foot region and rapidly transforms into a sporocyst (Toledo and Fried, 2011). During this stage, the parasite attempts to escape the internal defence system of the snail by modulating hemocyte activity (Núñez *et al.*, 1994; De Jong-Brink *et al.*, 2001). Nevertheless, hemocytes of resistant snails enclose the recently transformed sporocyst within their tissue and kill the parasite (De Jong-Brink *et al.*, 2001). In contrast, the response in susceptible snails is not adequate and in the next two to three weeks mother sporocysts generate clonal daughter sporocysts, still located in the head-foot region (Nacif-Pimenta *et al.*, 2012). Next, daughter sporocysts migrate to the gonad-digestive gland region to further multiply asexually, inflicting significant damage in the process (Faro *et al.*, 2013; Tavalire *et al.*, 2016). This damage leads to partial or complete destruction of

ovotestis tissue, typically castrating the snail host (Faro *et al.*, 2013; Tavalire *et al.*, 2016). Cercariae require arginine and glucose to mature within the daughter sporocyst thereby continuously absorbing large energy reserves of the gonad-digestive gland region during their development (Faro *et al.*, 2013). Combined, these processes reveal a direct (destruction of tissue) and indirect (snail starvation through nutrient absorption) effect on snail fitness (Faro *et al.*, 2013). However, once matured, cercariae require only a minimal supply of energy for migration and emergence which happens approximately four weeks after miracidial exposure of the snail (Faro *et al.*, 2013).

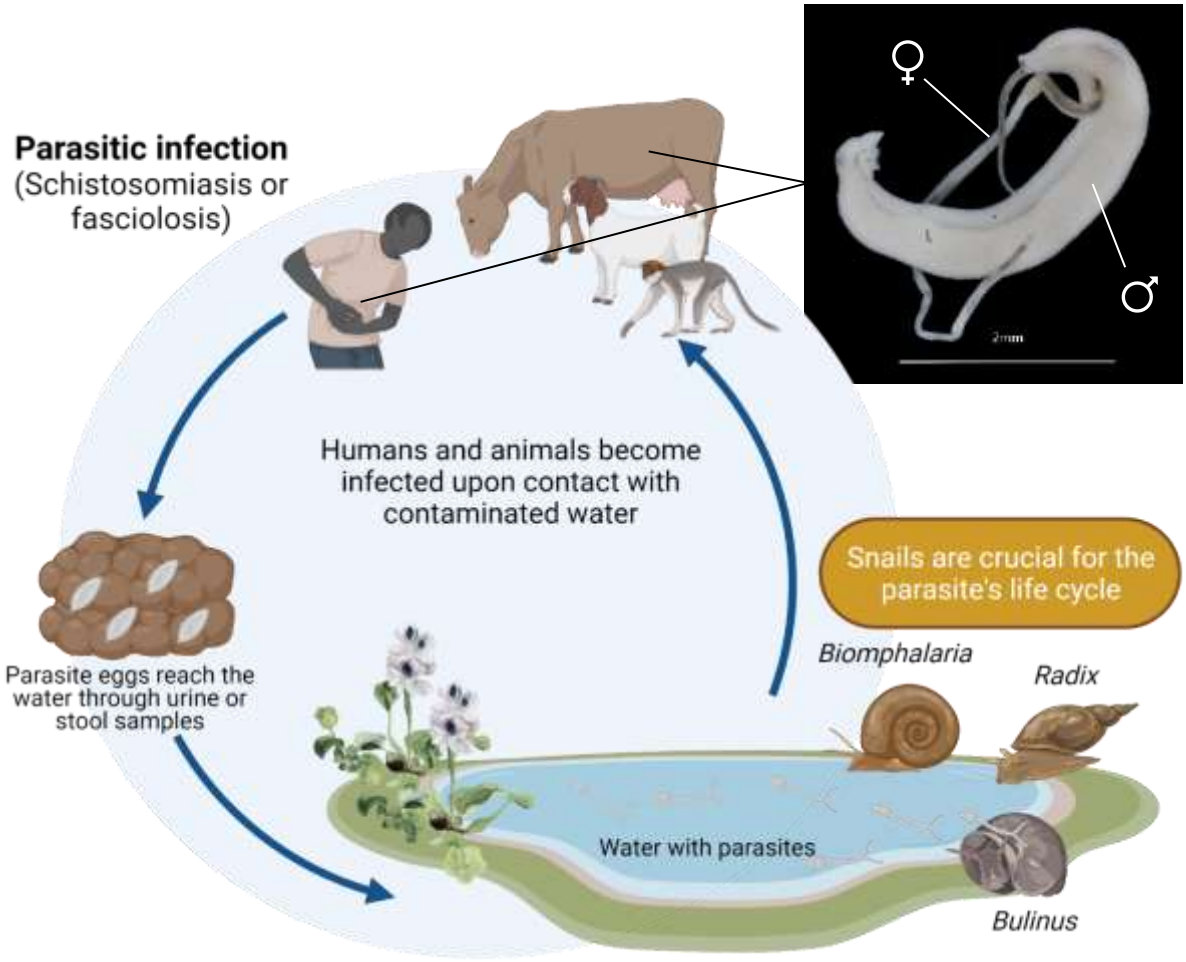

Figure 1: Generalized lifecycle for trematode parasites. Adult parasites in definitive hosts (human or animal) release eggs with excreta, from these larval stages called 'miracidia' hatch and infected snail intermediate hosts. Once infections have developed, the next larval stage 'cercariae' emerge from the snail and restart the cycle by infecting a new definitive host. Diseases as a consequence of infections with *Schistosoma* spp. or *Fasciola* spp. parasites are referred to as schistosomiasis and fasciolosis, respectively. Figure courtesy of Noelia Valderrama. Created with BioRender. Figure inset: *Schistosoma mattheei* collected by Schols R and Mudavanhu A from mesenteric veins of cattle in Zimbabwe. The larger male holds the smaller female in its gynecophoral canal.

Significant variations on this lifecycle exist with usually high host specificity and different levels of complexity, giving rise to the many species seen today and over 90% still waiting to be discovered (Littlewood *et al.*, 1999; Hechinger, 2023). Nevertheless, despite allowing many speciation events, the specialized and complex lifecycles also make most trematodes especially vulnerable to extinction (Cizauskas *et al.*, 2017; Wood *et al.*, 2023). Losing trematodes could have unexpected consequences as parasites can be part of up to three-quarters of food web links, constitute a major proportion of

ecosystem biomass, and regulate host populations (Lafferty *et al.*, 2006; Kuris *et al.*, 2008; Sato *et al.*, 2011). Consequentially, the idea of parasite conservation has been gaining traction (Cizauskas *et al.*, 2017; Carlson *et al.*, 2020; Kwak *et al.*, 2020; Milotic *et al.*, 2020), with the recent establishment of an IUCN SSC parasite specialist group as pinnacle (https://iucn.org/our-union/commissions/group/iucn-ssc-parasite-specialist-group). Nevertheless, certain trematodes do impose a severe economic and public health burden (Charlier *et al.*, 2014; WHO, 2023).

Trematode burden and control

The most impactful of these snail-borne diseases (SBD) is schistosomiasis. It is caused by blood flukes of the genus *Schistosoma* and affects over 200 million people globally with the largest burden in Sub-Saharan Africa (WHO, 2023). Some of the consequences of a schistosome infection include an increased chance of developing bladder cancer and contracting HIV while reducing fertility and causing kidney and liver disease (Forsyth and Macdonald, 1965; King and Dangerfield-Cha, 2008; WHO, 2016). Similarly, the second most impactful SBD, fasciolosis, is caused by liver flukes of the genus *Fasciola*. It infects an estimated 2.4 - 50 million people and millions more animals across all continents except Antarctica (Mas-Coma *et al.*, 2009a; Toledo and Fried, 2014; Nyindo and Lukambagire, 2015). Some consequences include severe liver disease and gastrointestinal pain in humans in addition to reduced milk production for dairy cattle (Schweizer *et al.*, 2005; Mas-Coma *et al.*, 2009a). With millions of people and animals suffering from SBDs, resulting in increased mortality and billions of economic losses, concerted and broadscale control efforts have been undertaken to this date (Wright, 1972; Charlier *et al.*, 2014; Sokolow *et al.*, 2016; WHO, 2016, 2023; Mehmood *et al.*, 2017; Mutapi *et al.*, 2017).

Control measures can target any step involved in the complex life cycle of schistosomes (**Figure 2**) (Mahmoud, 2001; Sokolow *et al.*, 2016). Firstly, the availability of clean drinking water and awareness raising prevents exposure to contaminated waters and hence the infective stage, cercariae. Preliminary data indicates that soap might offer a certain degree of protection against cercariae, but further research is required before it can be used in control efforts (Lemma, 1970; Zhang *et al.*, 2022). The natural soap obtained from the endod soapberry, *Phytolacca dodecandra,* seems to be the most promising and sustainable option (Lemma, 1970; Erko *et al.*, 2002; Abebe *et al.*, 2005). Nevertheless, clean drinking water is frequently unavailable or water contact is required for food collection hence infections still occur. Currently, no effective vaccine against schistosomiasis infection exists (Molehin, 2020; WHO, 2020). Therefore, curative treatments led by the drug Praziquantel remain the golden standard to treat human populations (Mutapi *et al.*, 2017). It is a highly effective and affordable chemotherapeutic drug (Kokaliaris *et al.*, 2022), that has extended its use to 250 million tablets being distributed for mass-drug administration programmes across the disease's range (Mutapi *et al.*, 2017; WHO, 2020). Nevertheless, it does not prevent re-infection nor is it effective against juvenile worms (Chandiwana *et al.*, 1991; Pica-Mattoccia and Cioli, 2004). To avoid open defecation and prevent eggs from entering the environment, proper sanitation and awareness are required, which are often lacking (Anyolitho *et al.*, 2022). Recently, control efforts are steering away from the typical top-down approach and are adopting bottom-up structures through citizen science, which empower communities and improve behavioural change to control the disease (Ashepet *et al.*, 2021; Anyolitho

et al., 2024; Tumusiime *et al.*, 2024). Simultaneously, citizen scientists can help attain broad-scale snail monitoring datasets, contributing to more efficient snail control measures which is critical for schistosomiasis control (Sokolow *et al.*, 2016).

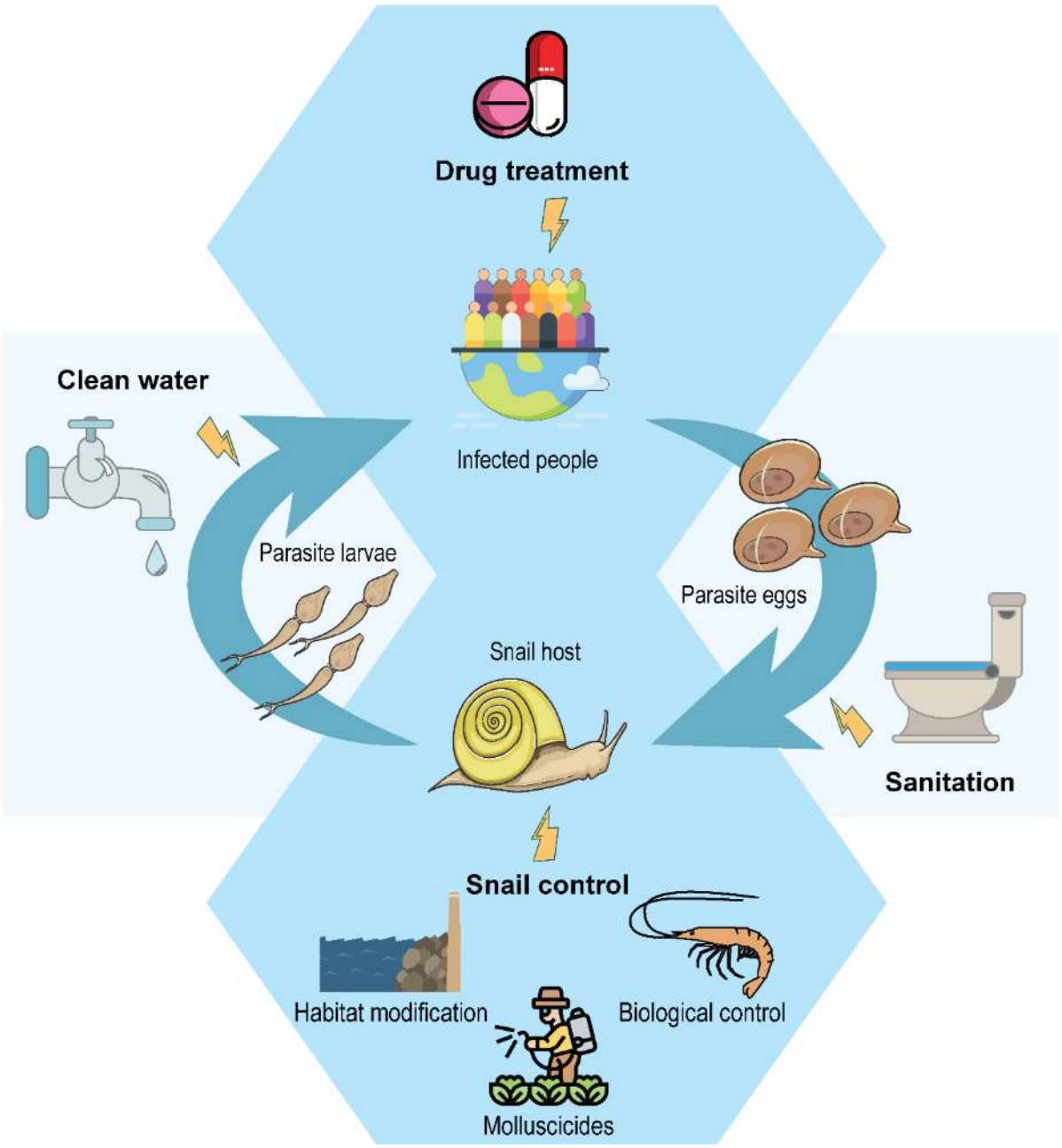

Figure 2: Current schistosomiasis control measures targeting the different schistosome life stages. Infected people can be treated with medicine such as Praziquantel. Parasite eggs can be prevented from reaching the environment with reliable sanitation. Snail control aimed at reducing snail numbers is currently conducted through habitat modification, applying molluscicides, or biological control. Contact with the infectious stage, cercariae, can be prevented by providing reliable and clean drinking water. Figure was constructed based on the scheme shown in Sokolow *et al.* (2016).

Today snail control depends on the use of chemical molluscicides, habitat modification and biological control. These chemicals theoretically only target snails, however, in field settings frequently lead to mass mortality in other aquatic life such as fish and amphibians and cause concern for human health

(Dai *et al.*, 2008; Sokolow *et al.*, 2016). Habitat modification involves the removal of aquatic vegetation, the main substrate for snails, which in turn may be used as cattle feed or as a soil fertilizer and boost crop yield (Rohr *et al.*, 2023). The water contact associated with such removal, a key transmission factor for schistosomiasis, makes protective gear imperative. Other habitat modifications such as irrigation schemes and artificial lakes can set the stage for unexpected epidemics (Picquet *et al.*, 1996; Grabner *et al.*, 2014; Sokolow *et al.*, 2017; Carolus *et al.*, 2019). Biological control has been tried through snail predators and competitors yet can have unexpected impacts on the ecosystem (Howarth, 1991; Appleton *et al.*, 2009; Ip *et al.*, 2014; Van Bocxlaer *et al.*, 2015; Sokolow *et al.*, 2016). Despite extensive control efforts to date, schistosomiasis continues to (re-)emerge across and beyond its distribution range with startling intensities (Picquet *et al.*, 1996; Boissier *et al.*, 2016; Sokolow *et al.*, 2018). In a bid to eliminate schistosomiasis as a public health concern by 2030, the WHO expressed a pressing need to improve our scientific understanding and create affordable and accurate diagnostic tools for effective disease interventions (WHO, 2020).

Trematodiases – complicating factors

Human impacts can manifest through globalization, climate change, pollution, and land use change. Increased connectivity through globalisation facilitates the spread of invasive species (Hulme, 2009) and presumably aided the introduction of Senegalese schistosomes in Corsica, France (Boissier *et al.*, 2016). Moreover, climate change-associated temperature rise is expected to increase distribution ranges throughout Europe of *Bulinus truncatus,* and thereby urogenital schistosomiasis since the parasite already tolerates these lower temperatures (Mulero *et al.*, 2019; De Leo *et al.*, 2020; Maes, 2024). Besides climate change, pollution and eutrophication could further increase SBD incidence. To meet food requirements intensified agriculture is required throughout developing areas (Godfray *et al.*, 2010), and consequentially environmental agrochemical concentrations are expected to increase (Snyder *et al.*, 2018; Ciceri and Allanore, 2019). Most of these agrochemicals have been linked to increased snail populations by, for example, eliminating predators or increasing the algal food source (Halstead *et al.*, 2018). Consequentially, models predict up to three times higher reproductive numbers for *S. haematobium*, leading to a much higher burden on the human population (Hoover *et al.*, 2020). The effect of agrochemicals is not limited to humans, as increased trematode infections could in part explain amphibian decline (Rohr *et al.*, 2008). Water availability is also crucial for agriculture and combined with the need for hydropower, has been the driver for dam construction and artificial lake creation (Tortajada *et al.*, 2012; De Leo *et al.*, 2020). Nevertheless, blocking natural water flow and creating stagnant waters comes at a cost (McAllister *et al.*, 2001). SBDs have increased on many occasions due to the shift in the aquatic ecosystem, interrupted migration routes of fish and crayfish, and facilitated biological invasions associated with the creation of artificial lakes (Talla *et al.*, 1990; Havel *et al.*, 2005; Sokolow *et al.*, 2017; Carolus *et al.*, 2019).

This non-exhaustive list of anthropogenic impacts underlines the need for a *One Health* framework for SBDs whereby human and environmental health are intertwined (Degeling *et al.*, 2015; Webster *et al.*, 2016; Gower *et al.*, 2017). Moreover, it reveals how complex SBDs truly are, potentially explaining their persistence and (re-)emergence (Braks *et al.*, 2014; Toledo and Fried, 2014; Boissier *et al.*, 2016; Sokolow *et al.*, 2016; Webster *et al.*, 2016). One such complicating factor is hybridization.

It is frequently reported for schistosomes and fasciolids and can result in hybrid vigour and broader host ranges (Wright and Ross, 1980; Morgan *et al.*, 2003; Hoa Le *et al.*, 2008; Huyse *et al.*, 2009; Boissier *et al.*, 2016; Nguyen *et al.*, 2018). This increased host range further complicates the already existing animal reservoirs and zoonotic tendencies of the disease (Huyse *et al.*, 2009; Toledo and Fried, 2014; Webster *et al.*, 2016; Gower *et al.*, 2017; Leger and Webster, 2017; You *et al.*, 2018; Alba *et al.*, 2021). These non-human sources of infection opened the discussion about treating animal reservoirs (Gower *et al.*, 2017), although they may increase the establishment of drug resistance (Webster *et al.*, 2016). For human populations, warnings on schistosome resistance to praziquantel have frequently emerged however remain controversial (Nuno *et al.*, 2017). Nevertheless, resistance to other drugs such as oxamniquine is widespread, revealing a cause for concern (Chevalier *et al.*, 2019). Notably, animal treatments for other helminths such as liver flukes do occur, yet targeted triclabendazole treatments against *Fasciola hepatica* are hypothesized to be partially at the basis of increased stomach fluke infections by *Calicophoron daubneyi* in European cattle by shifting intra-snail competition (Rondelaud *et al.*, 2007, 2022; Jones *et al.*, 2017). Similarly, Laidemitt *et al.* (2019) showed that within the snail *Biomphalaria pfeifferi*, the larvae of the amphistome *Calicophoron sukari* antagonize the development of *Schistosoma mansoni* yet require the latter for development within the snail host. Clearly, parasite-parasite interactions within a snail host can have a great influence on human and animal SBD epidemiology (Laidemitt *et al.*, 2019). Nevertheless, studying these parasite interactions remains challenging as species identifications are hindered due to a lack of taxonomists and reference sequences (Brant *et al.*, 2006; Schols *et al.*, 2020; Vinarski, 2020).

Similarly, some snail groups remain hard to identify due to cryptic species and species complexes despite the importance of accurate snail identification in assessing snail-parasite compatibility (Mukaratirwa *et al.*, 1998; Schniebs *et al.*, 2016; Babbitt *et al.*, 2023). Notably, such compatibilities go deeper than the species level, with dramatic differences at the population level within species. Noteworthy examples exist for fasciolosis and schistosomiasis. *Pseudosuccinea columella* transmits *Fasciola* spp., causative agents of fasciolosis or liver rot in wildlife, livestock and human populations (McCully *et al.*, 1967; Toledo and Fried, 2014). It is one of the major vector snails of *F. hepatica* (Krull, 1933; Dar *et al.*, 2015; Alba *et al.*, 2019) worldwide and increases the transmission of *F. nyanzae* (Carolus *et al.*, 2019; Schols *et al.*, 2021) and *F. gigantica* (Grabner *et al.*, 2014). Interestingly, certain naturally occurring *P. columella* populations in Cuba show complete resistance to *F. hepatica* infections, contrasting the high susceptibility of the globally distributed genotype (Alba *et al.*, 2022). After the construction of the Diama dam to embank the Senegal River Basin (SRB) in 1988, an important outbreak of urinary schistosomiasis rapidly ensued (Southgate *et al.*, 2000; Meurs *et al.*, 2012). This form of the disease is caused by *S. haematobium*, which uses *Bulinus globosus* and *Bulinus truncatu*s as intermediate hosts. In the lower part of the valley, *B. globosus* acts as the main snail host for *S. haematobium*, whereas *B. truncatus* does not transmit local *S. haematobium* strains (Picquet *et al.*, 1996; Sène *et al.*, 2004). In the upper valley, however, the situation is reversed and *B. truncatus* is the main host for *S. haematobium*, whereas *B. globosus* is not involved in transmission (Southgate *et al.*, 2000; Sène *et al.*, 2004). Clearly, biological interactions and human impacts play critical roles in the transmission of debilitating SBDs.

Hosts and parasites are in an evolutionary arms race, potentially leading to frequency dependent selection (Decaestecker *et al.*, 2007; Wolinska and King, 2009; Decaestecker and King, 2019). Specifically, parasites will evolve to infect the dominant host genotype, thereby, facilitating the cohabitation of multiple host genotypes in what is called the Red Queen hypothesis (Jokela *et al.*, 2009). The snail-trematode interaction has strong reciprocal selection as snails become castrated upon successful infection or trematodes are destroyed upon failed infection (King *et al.*, 2011; Decaestecker and King, 2019). Similarly, this hypothesis explains why trematode parasites enable sexually reproducing snails to co-exist with asexually reproducing snails, despite the latter having a much higher reproductive potential (Jokela *et al.*, 2009). However, the environment shapes host-parasite interactions thereby altering the strength and direction of selection (Wolinska and King, 2009). The confounding effect of environment on host-parasite coevolution may explain why Red Queen dynamics are so difficult to detect in the wild (Wolinska and King, 2009), but not impossible (Decaestecker *et al.*, 2007; Jokela *et al.*, 2009). One such confounding factor is the microbiome, which can mediate host-parasite coevolution (Decaestecker and King, 2019). As a result, recent works have proposed that the snail-associated microbiome might be a sustainable and specific target for SBD control (Portet *et al.*, 2021; Le Clec'h *et al.*, 2022; Sun *et al.*, 2024).

2. STATE OF THE ART AND OBJECTIVES

Microbiome - Definitions and Importance

The microbiome is defined as the combination of the microbiota – all living organisms: archaea, fungi, algae, protists, and bacteria – and all of their molecules – including metabolites and structural elements (Berg *et al.*, 2020). Phages, viruses and other mobile genetic elements are, therefore, considered part of the microbiome but not the microbiota, as they are not considered living organisms (Berg *et al.*, 2020). Macro-ecological effects such as the effect of grazing and predation also present at the microscopic scale. Bacterial communities themselves can be top-down regulated by protist predators, leading to increased bacterial diversity which in turn stabilizes predator performance (Corno *et al.*, 2008; Saleem *et al.*, 2013; Mosca *et al.*, 2016). These studies touch upon the complexity of the microbiome, yet these effects can extend to multicellular lifeforms. Some consider the human microbiome our latest organ under study (Baquero and Nombela, 2012), with the number of bacterial cells rivalling that of human cells (Sender *et al.*, 2016). The human-associated microbiome contains an estimated 250,000 unique genes, vastly exceeding the 20,500 unique genes in the human genome (Zilber-Rosenberg and Rosenberg, 2008).

The microbiome's influence on metazoan biology has become fully established over the last decade (Margulis, 1991; Rohwer *et al.*, 2002; Theis *et al.*, 2016; Berg *et al.*, 2020; Destoumieux-Garzón *et al.*, 2024). The importance of endosymbionts in the evolution of eukaryotic organisms was first raised by Margulis in 1991. In 2002 a new term was coined by Rohwer and colleagues – *the holobiont* – which refers to the intimate link between, and thus the unity of, a host and its microbiome, of which the latter constitutes a rapidly evolving aspect (Zilber-Rosenberg and Rosenberg, 2008; Alberdi *et al.*, 2016; Theis *et al.*, 2016; Bisschop *et al.*, 2022). The genome of the holobiont, or hologenome, consists of the genome of a host and the genomes of the host-associated microbiome (**Figure 3**) (Theis *et al.*, 2016). Notably, microbial genes in the holobiont can directly affect host phenotype, not affect host

phenotype, or coevolved with host genes to affect the host phenotype. In contrast, environmental microbes are not part of the holobiont yet they can become part of it in the future (Theis *et al.*, 2016).

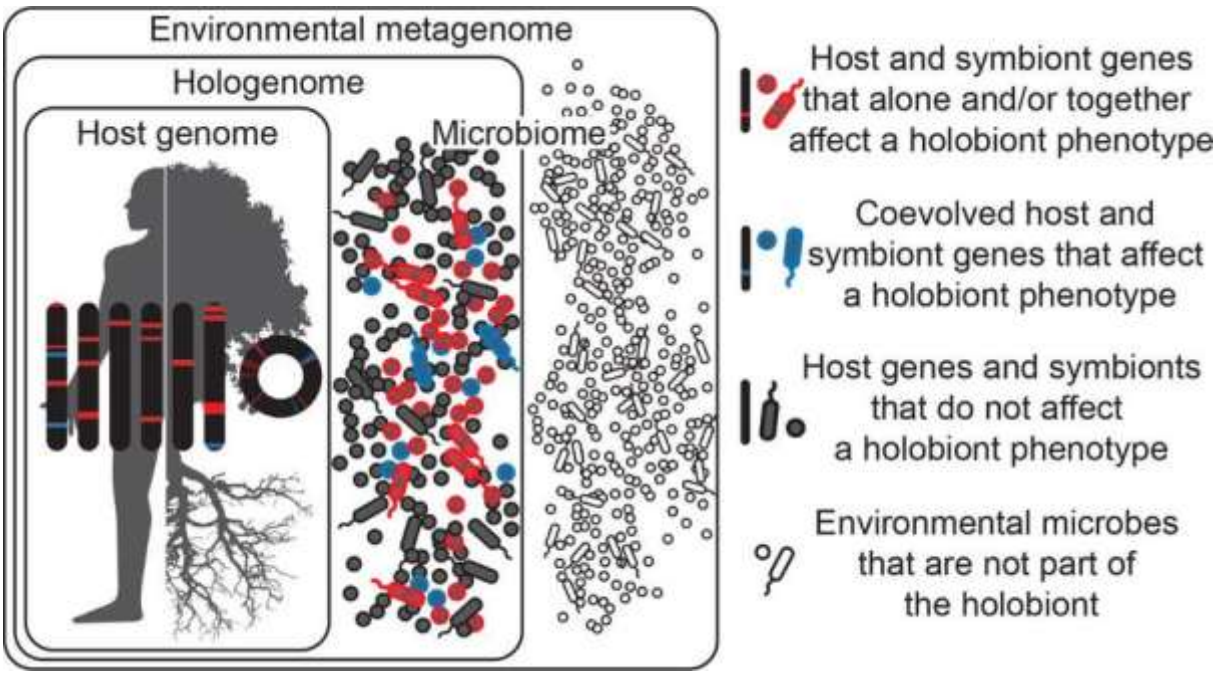

Figure 3: Schematic overview of the hologenome and environmental bacteria (white) as represented in Theis *et al.* (2016). Host and symbiont genes can either directly shape host phenotype (red), have coevolved to shape the host phenotype (blue), or do not affect the host phenotype (dark grey). The hologenome consists of the host genome and the genomes of all symbionts. The figure was reproduced from Theis *et al.* (2016).

Microbial symbiosis usually refers to a larger unicellular or multicellular organism or host colonized by a unicellular microorganism or symbiont (Stock *et al.*, 2021). These microorganisms occur at various sites of a host ranging from the epithelial surface to specialized organs called bacteriocytes (Zilber-Rosenberg and Rosenberg, 2008). The symbiotic interactions can be strong, weak, beneficial, negative or neutral and can change depending on the circumstances (Daskin and Alford, 2012). The endosymbiotic algae providing nutrients while living within corals might be the most well-known mutually beneficial symbiosis (Yellowlees *et al.*, 2008). Another frequently used example is the collaboration between squids and *Vibrio fischeri*, whereby the bacterium resides in a specialized light organ within the squid allowing hosts to avoid casting a shadow and thus remain camouflaged (Dunlap *et al.*, 2007). Within the molluscs, deep-sea mussels have symbiotic sulphur- and methane-oxidizing bacteria which also protect them from parasitic bacteria infecting the host clam nucleus and feeding on host chromatin (Zielinski *et al.*, 2009). Plant-sap-feeding insects in general are highly dependent on their endosymbionts to obtain sufficient nutrients due to their monotonous and nutritionally simple food sources (Douglas, 2009). However, not all insects depend on symbionts as exemplified by the lack of resident gut bacteria in certain caterpillars (Hammer *et al.*, 2017). Others only depend on endosymbionts when it suits them, cereal-feeding weevils need the *Sodalis pierantonius* symbiont to be able to make a strong cuticle yet subsequently eliminate this bacterium to avoid costs associated with the symbiosis (Dell'Aglio *et al.*, 2023). In contrast to the host ending the mutually beneficial interaction, the deviation may also come from the bacteria's side. Opportunistic pathogens are perhaps the most renowned, whereby usually commensal bacteria and fungi can cause deadly

infections in people with a weakened immune system (Overstreet and Lotz, 2016). The shift in pathogenicity can also originate from an environmental trigger whereby a change in, for example, temperature can drastically alter the virulence of the amphibian disease chytridiomycosis (Daskin and Alford, 2012). Mounting evidence points to a shaping effect of human-induced environmental change on the host-associated microbiome (**Figure 4**) (Stock *et al.*, 2021). For example, eutrophication leads to dysbiosis in the skin microbiota of fish (Krotman *et al.*, 2020), antibiotic use to a shift in microbial ecological interactions in water fleas (Callens *et al.*, 2018), and global warming to altering microbial metabolism in the gut of tadpoles (Fontaine and Kohl, 2020). In contrast, the microbiome itself can modify host phenotypes and thereby ultimately affect ecosystem functioning (Moreira *et al.*, 2009; Koch and Schmid-Hempel, 2012; Ridaura *et al.*, 2013; Macke *et al.*, 2017a) through adaptations in for example, host physiology (Sudo *et al.*, 2004), behaviour (Desbonnet *et al.*, 2014), and disease resistance (Harris *et al.*, 2009; Lee and Mazmanian, 2010; Graça *et al.*, 2013; McLaren and Callahan, 2020). As mentioned earlier, snails are key members of the aquatic ecosystem (Brönmark, 1989), upon recognition of their importance, scientists have been expanding their efforts to better understand their microbiome.

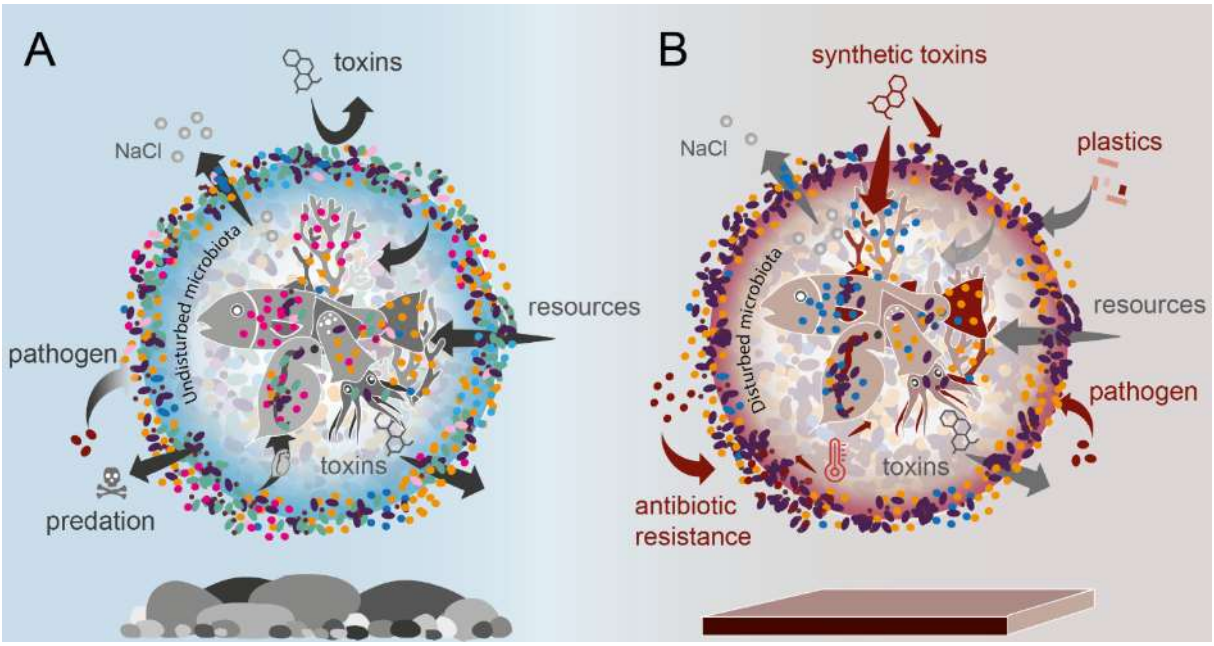

Figure 4: Overview of benefits provided to a host through microbial symbionts and how anthropogenic influences could alter these interactions. A) Beneficial services under natural conditions: Protection against toxins, pathogens and predation, osmoregulation, resource uptake, host development, providing metabolites, and the production of toxins. B) (red arrows) Human-induced disruption of the microbiome can happen through climate change, antibiotic resistance, toxins, pathogenic organisms, and microplastics. (grey arrows) Disturbed microbiota can affect the host through reduced toxin production or metabolisation, reduced resource acquisition, reduced pathogen resistance, and ineffective osmoregulation. Figure reproduced from Stock *et al.* (2021) with courtesy of Ester M. Eckert.

Snail microbiome

The repertoire of microbiome studies in freshwater snails is limited, therefore, we will occasionally also include studies on slugs and the related class Bivalvia. Investigations on the microbiome of freshwater snails started in the mid-20th century. Chernin and colleagues pioneered the field with the

limited resources of the time, developing protocols to obtain axenic (bacteria-free) snails, maintaining them in culture and exposing them to schistosome parasites (Chernin, 1957, 1960; Chernin and Schork, 1959). Some decades later, the bacterial ecology of schistosome vector snails started receiving attention. These works revealed a 10-fold increase in bacterial cell count when snails were stressed or moribund, suggesting an interaction between the snail immune system and the microbiome, while also revealing the potential for opportunistic pathogens in this system (Ducklow *et al.*, 1979, 1981). Moreover, these earlier works already describe a limited effect of environmental bacteria on the snail microbiome, which is confirmed by recent studies using more sensitive 16S rRNA gene metabarcoding to characterize the bacterial community (Chevalier *et al.*, 2020; Huot *et al.*, 2020). However, this is not a ubiquitous pattern across molluscs as the microbiome of the invasive snail *Potamopyrgus antipodarum,* the invasive bivalve *Corbicula fluminea,* and the snail *Galba truncatula* are affected by the environment (Bankers *et al.*, 2021; Chiarello *et al.*, 2022; McCann *et al.*, 2024). Moreover, a cellulose-rich or cellulose-poor diet modifies the gut microbiome in *Planorbella trivolvis* (Hu *et al.*, 2021), which could also be seen as an environmental effect. The gut microbiome changes throughout the gut and with snail host ageing in *Pomacea canaliculata* (Li *et al.*, 2019; Chen *et al.*, 2021). In the intertidal snail *Littorina saxatilis* different gut microbiome profiles are found depending on the ecotype and the vertical gradient on cliffs (Panova *et al.*, 2023). How far these patterns extrapolate across species is difficult to assess since the snails' phylogeny and their associated microbiome are congruent (Huot *et al.*, 2020), hence patterns could be species-specific.

Not all microbiota are beneficial or neutral for snails. Several examples exist of microbial pathogens of snails yet bivalves have had several reports of DNA-eating bacteria in deep-sea mussels (Zielinski *et al.*, 2009), viral disease in Chinese pearl mussels (Grizzle and Brunner, 2009), and larval mycosis caused by an oomycete infection in Eastern oysters (Grizzle and Brunner, 2009). Limited diseases are reported in freshwater bivalves presumably due to their highly efficient sorting of unwanted particles from their filtrate and sensitive differentiation up to cyanobacteria strain level (Grizzle and Brunner, 2009). Cole and colleagues did provide the first report of a bacterial infection in the freshwater snail *Bulinus jousseaumei* (Cole *et al.*, 1977), which, despite causing nodules in the snail tissue, did not increase mortality nor reduce fertility. More recently, however, *Paenibacillus glabratella* was isolated and described from nodules in *B. glabrata* (Duval *et al.*, 2015). This pathogen is both vertically and horizontally transmitted to other snail individuals, killing the new host within 30 days.

Microbiome transmission mostly occurs between snail individuals horizontally, but vertical transmission also occurs (Lin *et al.*, 2023) albeit not within snail eggs for *B. glabrata* (Schols *et al.*, 2023). Across snail species *Cloacibacterium* sp. and *Aeromonas* sp. have frequently been reported as members of the core microbiome, suggesting cross-generational transmission or consistent re-assimilation from the environment (Lin *et al.*, 2023). This suggests the importance of pseudo-vertical transmission - the indirect transfer of microbiota from parent to offspring through for example feces or nursing (Alberdi *et al.*, 2016)- in this system. Moreover, the reproductive mode as well as trematode resistance of *P. antipodarum* are thought to be linked to the microbial symbionts (Takacs-Vesbach *et al.*, 2016).

Trematode microbiome

Better understanding the microbial symbionts of parasitic organisms might be key to understanding the varying degrees of parasite virulence and transmission, as the microbiome can modify host phenotypes. Indeed, RNA viruses have been reported to increase disease pathology, parasite load, and drug resistance of infections by protozoan *Leishmania* species (Ives *et al.*, 2011). Moreover, increased parasite gene flow and hybridization events seem to stimulate the frequency of this parasite-virus symbiosis (Heeren *et al.*, 2023). Hybridization frequently occurs in both free-living and parasitic flatworms (Théron, 1989; Agatsuma *et al.*, 2000; Henrich *et al.*, 2013; Webster *et al.*, 2013; Singh *et al.*, 2020). Notably, the virome differs between free-living and parasitic flatworms, on most occasions co-diversified with the parasite, and most likely led to the emergence of some vertebrate-associated viruses (Hahn *et al.*, 2020; Dheilly *et al.*, 2022). For now, though, it remains unclear how such viral associations might affect the outcome of flatworm infections.

The field of trematode microbial symbionts is in its infancy, yet some notable patterns were already discovered. The first description of bacteria closely associated with a trematode was made in 1973 by Morris, describing a bacterial species strongly attached to the tegument of the trematode *Megalodiscus temperatus*, which infects frogs (Morris, 1973). The function remains unknown to this date yet trematode tegument plays a crucial role in avoiding detection by the host's immune system (Mahmoud, 2001), suggesting a potentially relevant role for bacteria in parasite camouflage. *Neorickettsia* are non-obligate intracellular bacteria of trematodes that can jump to vertebrate hosts and cause disease (Toledo and Fried, 2014; Fischer *et al.*, 2017). These bacteria have been shown to occur throughout various tissues in their trematode hosts, which suggests the potential for vertical transmission and horizontal transmission to other trematodes through mating and as mentioned earlier to the vertebrate host (Fischer *et al.*, 2017).

Similarly, parasitic flatworms maintain parts of their microbiome (bacterial and viral component) throughout their lifecycle and various hosts but also possess microbial components unique for each stage and associated host (Hahn *et al.*, 2020; Jorge *et al.*, 2020). This indicates the potential for co-evolution between the trematode and its microbiome (Jorge *et al.*, 2020). In contrast to the phylosymbiosis pattern reported for freshwater snails (Huot *et al.*, 2020), no such pattern appears visible for trematode parasites (Salloum *et al.*, 2023b). Furthermore, trematodes co-infecting a snail host mostly have a unilateral bacterial exchange (Salloum *et al.*, 2023b). Some trematodes can cut their complex multi-host lifecycle short by asexually reproducing within an intermediate host, following that of the first intermediate snail host (Salloum *et al.*, 2023a). Preliminary results indicate a potential yet weak link between this alternative life cycle and the relative abundance of certain taxa but not the diversity of the microbiome (Salloum *et al.*, 2023a). Similar to social bees, certain trematodes are considered social and have a caste system whereby soldiers protect the trematode against other trematodes and reproducers ensure parasite reproduction and transmission (Poulin *et al.*, 2019). Bacterial communities were shown to differ between both castes whereby the community level is potentially driving the caste classification as no single bacterial taxon was prevalent enough to be indicative of a caste (Jorge *et al.*, 2022a). Moreover, AB exposure would lead to increased proportions of reproducers compared to soldiers, suggesting a possible causative relationship

between the microbiome and caste (Jorge *et al.*, 2022a). However, no direct relationship could be shown and similarly exposing the snail host to antibiotics has been indicated to cause a shift in the post-exposure trematode microbiome compared to the pre-exposure microbiome (Jorge *et al.*, 2022b). Potentially, also, a tripartite interaction could be at play with the snail host, trematode parasite and their microbiomes all interacting to determine infection outcome (Le Clec'h *et al.*, 2022), in line with disease resistance reported elsewhere (Harris *et al.*, 2009; Lee and Mazmanian, 2010; Graça *et al.*, 2013; McLaren and Callahan, 2020).

Tripartite interactions

The pathogen-host interaction can have major consequences for the outcome of subsequent infections. For example, in humans and lab models infections with *Schistosoma* spp. can occasionally lead to less severe subsequent infections resulting in, for example, a reduced *Fasciola* spp. worm burden and reduced gastrointestinal complaints associated with *Helicobacter pylori* infections (Abruzzi and Fried, 2011). However, in most cases, prior *Schistosoma* infections increased the severity of subsequent infections. For example, *Salmonella* infections were more persistent, more virulent and caused higher host mortality while *Staphylococcus aureus* infections were linked to higher counts of liver abscesses (Abruzzi and Fried, 2011). However, the host-associated microbiome can also influence host physiology and disease outcome (Ford and King, 2016; Brinker *et al.*, 2019; Greyson-Gaito *et al.*, 2020). The associated microbiota of aquatic organisms often plays a crucial role in the host's resistance or tolerance to pathogen infection (Kimura and Tomaru, 2014; Dheilly *et al.*, 2015; Greenspan *et al.*, 2019; Davoodi and Foley, 2020; Schellenberg *et al.*, 2020; Portet *et al.*, 2021). For instance, Huot *et al.* (2020) reported a congruence of vector snails' phylogeny and their associated microbiome, combined with variable susceptibility between snail species and even populations of the same species, suggesting that the snail's microbiome might play a role in parasite resistance. Microbiota-induced pathogen resistance or tolerance has three underlying mechanisms: competition for resources and space, production of antimicrobial substances and stimulation of the host's immune response (Dheilly *et al.*, 2015; Mallon *et al.*, 2015; Knutie *et al.*, 2017).

It is worth noting that the diversity of the microbiota is often positively correlated with colonization resistance, although a specific mechanistic explanation is often lacking. On the one hand, such a pattern could be caused by competition, as communities with high species richness occupy more niches and hence are more resistant to invading pathogens by leaving less space and nutrients available for the pathogen to exploit (Mallon *et al.*, 2015). On the other hand, it could be that key species providing resistance (e.g. through the production of antimicrobial substances) are often missing in less diverse communities. A positive correlation between microbiota diversity and colonization resistance was observed in the European common frog *R. temporaria*, which showed increased resistance to the emerging Ranavirus with a more diverse skin microbiome (Harrison *et al.*, 2019). When the microbiota diversity in zebrafish is reduced through exposure to the antibiotic olaquindox, they also become more susceptible to infection by the pathogen *Aeromonas hydrophila* (He *et al.*, 2017).

Consequentially, the vector-associated microbiome may indirectly drive the health of definitive hosts by determining the vector's transmission potential (Hoffmann *et al.*, 2011; Pinto *et al.*, 2021). Indeed,

the prevalence of mosquito-borne diseases like malaria, dengue, and chikungunya have all been reduced by releasing microbiome-altered mosquito vectors, which have mostly been targeting the *Wolbachia* endosymbiont (Moreira *et al.*, 2009; Hoffmann *et al.*, 2011; Pinto *et al.*, 2021). This reduction in transmission can occur due to various microbiome-mediated effects such as: reducing the host's lifespan (McMeniman and O'Neill, 2010), stimulating the mosquito's immune response (Bai *et al.*, 2019), producing anti-pathogen proteins (Wang *et al.*, 2017), and supporting the formation of physical barriers (Song *et al.*, 2018). However, it is not a one-shoe-fits-all system as several studies have since reported an increase in pathogen transmission associated with *Wolbachia* infections in mosquitos (Hughes *et al.*, 2012; Zélé *et al.*, 2014). These conflicting results indicate a complex tripartite interaction between vectors, their microbiome and vector-borne diseases, which can change depending on the interacting species and strains (Moreira *et al.*, 2009). Nevertheless, combined with a fundamental understanding of all the epidemiological drivers across a disease's range, microbiome-mediated vector-borne disease control provides an exciting and promising avenue for future disease control efforts.

The snail's immune response to infection is influenced by host genetic factors (Allan *et al.*, 2018) and by abiotic environmental factors like temperature (Leicht *et al.*, 2013; Knight *et al.*, 2015). Resistance can artificially be induced by stimulating the snail's hemocytes (Pila *et al.*, 2016), which indicates the potential for modulating the snail immune response. Combined with the successes in mosquito-borne diseases, the idea of microbiome-mediated schistosomiasis control got a major boost after Chernin first touched upon the concept in 1957 (Chernin, 1957; Chevalier *et al.*, 2020; Huot *et al.*, 2020; Portet *et al.*, 2021; Le Clec'h *et al.*, 2022; Sun *et al.*, 2024). In 1960 Chernin showed that bacteriologically sterile (as determined by thioglycolate broth) *B. glabrata* were equally receptive to schistosome infection and survived better than an untreated strain of *B. glabrata*. Since then, no studies repeated Chernin's protocol and validated it with state-of-the-art and increasingly sensitive molecular tools. Moreover, once these protocols are validated it lays the foundation for microbiome transplant experiments whereby specific bacteria or bacterial communities can be actively or passively exchanged from one host or substrate to another (Greyson-Gaito *et al.*, 2020). Such transplant experiments disentangle causation from correlations and have shown that the microbiome is critical for toxic cyanobacteria tolerance in water fleas (Macke *et al.*, 2017a; Houwenhuyse *et al.*, 2021), for obesity in lab mice (Ridaura *et al.*, 2013), and for parasite resistance in bumblebees (Koch and Schmid-Hempel, 2012).

Altogether, field- and lab-based studies, including lab infection experiments, field-based correlative data, microbiome transplant experiments, and microcosm studies can converge to a fundamental understanding of all the involved epidemiological drivers such as complex compatibility patterns (Sène *et al.*, 2004) and parasite-parasite interactions (Laidemitt *et al.*, 2019). As a result, sustainable disease control will hopefully be possible through a nature-based solution, ensuring public, economic and environmental health as dictated by the *One Health* framework (Webster *et al.*, 2016).

The MicroResist project tackled the complex tripartite interaction between freshwater snails, trematode parasites, and the microbiome through four interconnected aims (**Figure 5**). The first aim

of the project targeted a lab-based infection experiment. This experiment was designed to understand what changes the snail microbiome goes through during the maturation of schistosome infections. Moreover, we aimed to understand what happens to the bacterial aspect of the microbiome during key parasite development stages, the various effects different schistosome species might have on the snail bacterial profiles, and to compare these effects under single parasite infections to co-infection conditions. The second aim of the project was to validate the axenic tool of Chernin (1957) and build upon its foundation to create a viable transplant protocol for the bacterial aspect of the microbiome of freshwater snails. The third aim of the project was to investigate a potential link between the bacterial aspect of the microbiome of a globally invasive snail and its resistance to trematode infection through a reciprocal transplant experiment. The final aim of the project steered away from the microbiome and zooms in on the anthropogenic pressures driving snail-parasite interactions in man-made lakes.

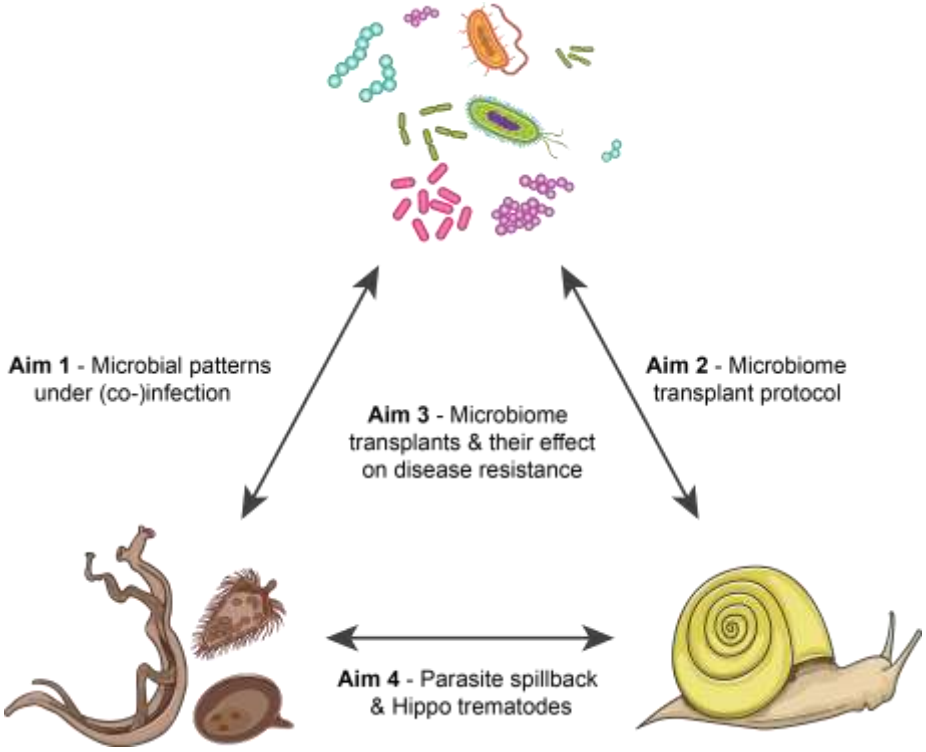

Figure 5: An overview scheme of how the MicroResist project approached the tripartite interaction between freshwater snails, trematode parasites and the microbiome. See the above text for details.

3. METHODOLOGY

Aim 1 – Microbial patterns under (co-)infection

The project began by targeting a lab-based infection experiment to understand how the snail microbiome changes during the maturation of schistosome infections. This included examining the bacterial aspect of the microbiome during key parasite development stages, assessing the effects of different schistosome species, and comparing single versus co-infection conditions in experimental infections using the lab model snail *Biomphalaria glabrata* (**Figure 6**). Briefly, laboratory-reared snails of *B. glabrata* population BgGUA2 of the same age cohort (5-7mm) from Guadeloupe, Dans Fond, were simultaneously exposed to various allopatric combinations of *S. mansoni* population BRE (Brazil,

Recife, low-shedder, from here on referred to as SmBRE), *S. mansoni* population LE (Brazil, Belo Horizonte, high-shedder, from here on referred to as SmLE), and *S. rodhaini* (Burundi, from here on referred to as Sr) at the Host-Pathogen-Environment Interactions laboratory of the University of Perpignan (UPVD, France). The snail and parasite populations have been in laboratory cycling for several decades (Theron *et al.*, 2014; Portet *et al.*, 2019; Hammoud *et al.*, 2022). Parasite populations have been maintained through Swiss OFI mice as final hosts and their sympatric snail intermediate host: SmBRE with *B. glabrata* population BRE, SmLE with *B. glabrata* population Barreiro and Sr with *Biomphalaria pfeifferi*. Miracidia were obtained from schistosome infections in the mouse strain Swiss OFI whereby eggs were isolated from the liver and allowed to hatch in water. Miracidia were allowed to infect individual snails for 24h. To address the three abovementioned objectives, four different sub-experiments were conducted. A first sub-experiment was performed to characterize the detectability of parasites during infection development, involving 144 snails each individually exposed to a single SmBRE miracidium where specimens were sacrificed 2, 6, 10, 16, 20, 30 and 40 days post-parasite exposure (Figure 1B). Another sub-experiment focused on the detectability of various parasite species, involving 30 snails individually exposed to 10 miracidia of either of the three schistosome populations and sample collection at 40 days post-parasite exposure (Figure 1C). Finally, two last sub-experiments aimed at characterizing co-infections by SmBRE and SmLE, and SmLE and Sr, involving 96 snails individually exposed to 10 miracidia of each parasite population and samples being collected at 2, 10 and 40 days post-parasite exposure (Figure 1D & E). Control snail specimens of the same age cohort were collected from the donor population on day 0 (n=5) and day 40 (n=5) of the experiment. Across sub-experiments, snails were maintained in a single aquarium per exposure experiment in 10L aquaria at 25°C under a 12h:12h light-dark regime with constant aeration. Snails were fed *ad libitum* with washed pesticide-free salad and water was replaced once a week with borehole water. Snails were killed by heat shock (70°C for 1 min) and stored in 80% ethanol.

Earlier work on these samples enabled detailed insights into parasite exposure and infection status through a high-throughput amplicon sequencing (HTAS) workflow developed in collaboration with colleagues from the Université Perpignan Via Domitia (UPVD, France) (Hammoud *et al.*, 2022). Microbiome profiling was performed using 16S rRNA gene metabarcoding targeting the V3–V4 regions. PCRs were run in triplicate, pooled, cleaned, and sequenced on the Illumina MiSeq platform in two separate runs. Mock communities were included to assess sequencing accuracy and determine filtering thresholds. Quantitative PCR (qPCR) was used to estimate bacterial load, with results compared across infection statuses. Sequencing data were processed using QIIME2 and R. Reads were quality-filtered, denoised, and merged into amplicon sequence variant (ASV) tables. Contaminants, chloroplasts, mitochondria, and low-abundance ASVs were removed. Rarefaction was applied to standardize sequencing depth. Alpha diversity (Shannon and Faith's PD) and beta diversity (Bray-Curtis, UniFrac) were analyzed using generalized linear models, NMDS, and RDA. Core microbiome analysis and Venn diagrams were used to identify shared taxa. A read-count threshold of four reads per sample was applied to exclude spurious ASVs.

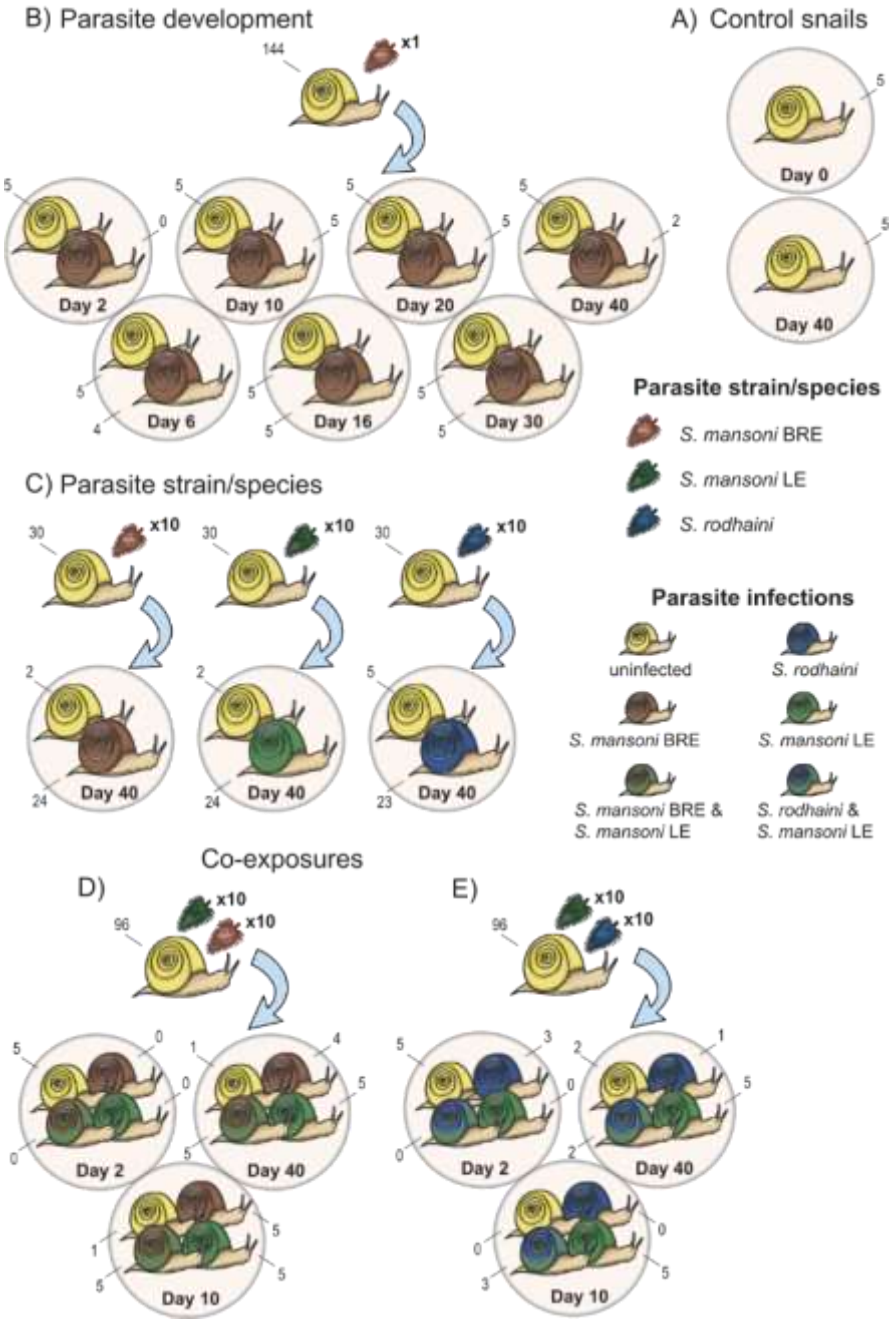

Figure 6: Experiment setup. Miracidia of *Schistosoma mansoni* population Recife (SmBre, low shedder, brown), Belo Horizonte (SmLE, high shedder, green), and *Schistosoma rodhaini* (blue) were obtained from experimentally infected mice. A) control snails were collected from the donor population at day 0 and day 40 of the experiment. B) *Biomphalaria glabrata* snails were exposed to single miracidia of *S. mansoni* strain Bre and sacrificed 2, 6, 10, 16, 20, 30 and 40 days post-miracidia exposure. C) *B. glabrata* snails were exposed to 10 miracidia of either *S. mansoni* Bre, *S. mansoni* LE or *S. rodhaini* and sacrificed 40 days post-miracidia exposure. D) *B. glabrata* snails were co-exposed to 10 miracidia of *S. mansoni* Bre and *S. mansoni* LE and sacrificed 2, 10 and 40 days post-miracidia exposure. E) *B. glabrata* snails were co-exposed to 10 miracidia of *S. mansoni* LE and *S. rodhaini* and sacrificed 2, 10 and 40 days post-miracidia exposure. Control snails were never exposed to miracidia and collected at day 0 and 40. Color codes of snail shells correspond to the infection status as determined by the HTAS protocol of Hammoud *et al.* (2022). The numbers on top (144 in B, 30*3 in C, 96 in D, and 96 in E) indicate the initial sample sizes, while the numbers showing the treatments are the final samples of which the bacterial component of the microbiome was characterized through 16S rRNA gene metabarcoding in this project.

Aim 2 – Microbiome transplant protocol

Building on this, the project focused on validating, optimizing, and expanding Chernin's (1957) protocol to establish a viable tool for microbiome transplantation in *B. glabrata*. This involved bacterial growth assays, quantitative PCR (qPCR), and microscopic investigations to determine the conditions under which snails could acquire new bacterial communities (**Figure 7**). Laboratory-reared *B. glabrata* (Brazil strain from UPVD) were maintained under standardized conditions and used to establish maternal lines. To create microbiome-disturbed individuals, snail eggs were surface-sterilized using a bleach protocol in a sterile biosafety cabinet. These germ-free snails were then exposed to microbiomes from three donor snail types: a different *B. glabrata* maternal line, *Planorbarius corneus*, and *Stagnicola fuscus × S. palustris*, representing increasing phylogenetic distance. The transplant experiment tested whether recipient snails could acquire donor microbiomes, how timing of exposure influenced colonization, and how phylogenetic relatedness affected outcomes. Donor inocula were prepared by homogenizing donor snails in autoclaved water. Each recipient group (three maternal lines, four exposure timings) received one of the three donor microbiomes, with positive and negative controls included. Survival was monitored, and snails were pooled for DNA extraction. Germ-free status was confirmed using a bacterial growth assay, fluorescence microscopy, and qPCR targeting the 16S rRNA gene. DNA was extracted using a mollusc-specific kit, quantified, and concentrated when necessary. Microbiome composition was assessed via 16S rRNA gene metabarcoding (V3–V4 regions), with sequencing performed on an Illumina MiSeq platform. Mock communities were included to assess bias and determine filtering thresholds. Sequencing data were processed using QIIME2 and R, including quality filtering, chimera removal, taxonomy assignment (SILVA database), and decontamination. Alpha and beta diversity metrics were calculated, and statistical analyses were performed to assess treatment effects. Core microbiome analyses and survival analyses were also conducted. A 0.5% relative abundance threshold was applied to remove spurious ASVs.

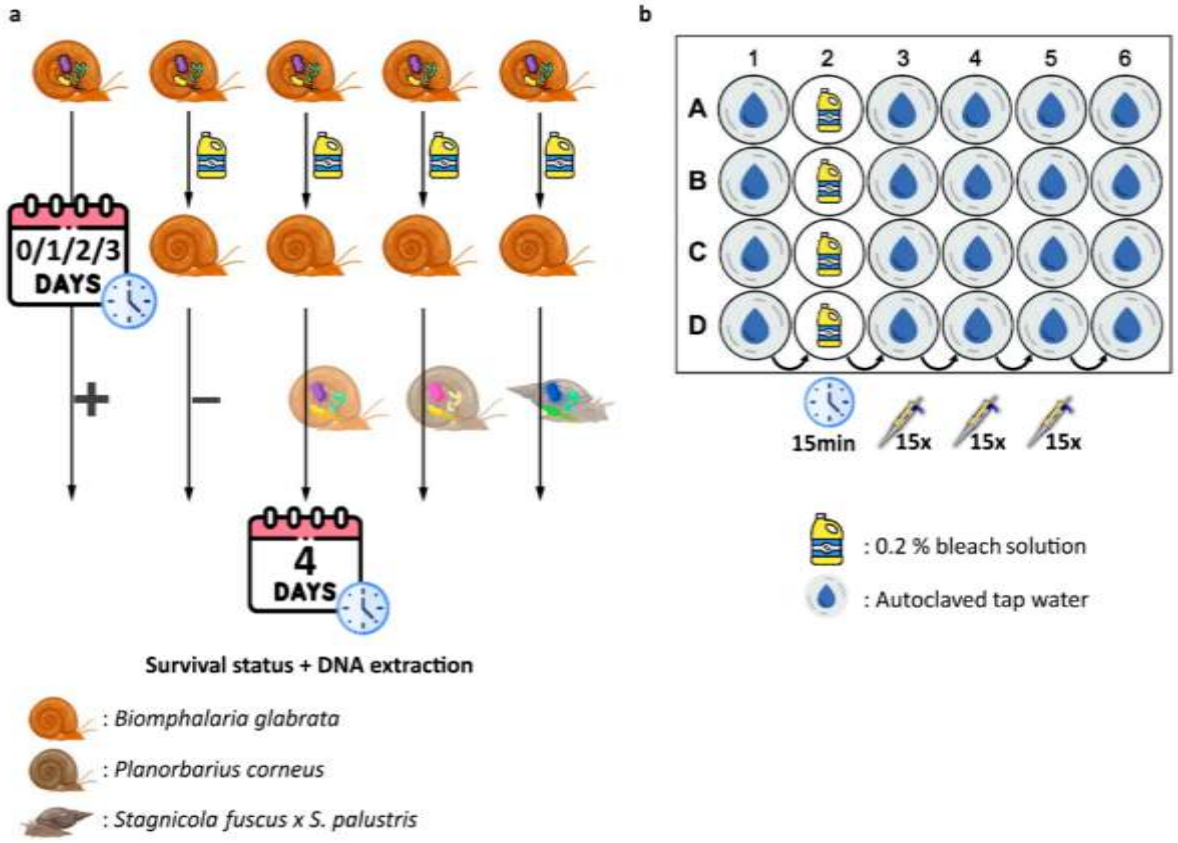

Figure 7: a) Experimental setup. Snails were sterilized on the same day as, and one day, two days and three days prior to, receiving a donor inoculum. Bacteriome-disturbed *Biomphalaria glabrata* individuals (n=8) of three maternal lines (n=3) were exposed to three donor inocula isolated from: *B. glabrata* individuals (same species but different maternal line), *Planorbarius corneus* individuals (family: Planorbidae), and *Stagnicola fuscus x S. palustris* individuals (superorder: Hygrophila). Additionally, for each factorial combination of time (n=4) and maternal line (n=3), positive (untreated samples) and negative (bleach-treated samples without donor inoculum exposure) controls were included (four technical replicates). Survival status of the egg/juvenile was noted on the day of the donor inoculum exposure, three days post donor inoculum exposure and four days post donor inoculum exposure when the specimens were sacrificed for DNA extraction. b) The 24-well cell culture plate setup used during the sterilization protocol. The wells of the first column receive one egg each, prior to bleach exposure. The egg from A1 is transferred to the well A2 which contains a 0.2% bleach solution and remains there for 15 min. After the 15 min bleach exposure, the egg was washed in autoclaved tap water in A3, A4 and A5 by pipetting up and down 15 times in each well before being moved to the incubation well A6. This process was done simultaneously for all four rows (A, B, C and D) for a total of four eggs per plate. The incubation time in column six depended on the assigned condition of that sample in accordance with **Figure 7a** (0, 1, 2, or 3 days) before being exposed to a donor bacteriome. The survival status of each specimen was noted, and irrespective of whether the specimen had hatched, still in the egg, or died, was exposed to the donor inoculum in their incubation well in column six.

Aim 3 – Microbiome transplants and their effect on trematode resistance

The project then applied this transplantation protocol to investigate whether the bacterial aspect of the microbiome plays a causative role in trematode resistance in the globally invasive snail *Pseudosuccinea columella* (**Figure 8**). The experiment was conducted at UPVD (France). The experiment consisted of six key milestones (**Figure 8**):

Step 1: individual eggs were dissected from egg clutches with the use of dissection needles under a dissection microscope. Several egg clutches would be dissected in a sterile petri dish containing sterile borehole water. The sterile water was replaced and the dissection needles were disinfected with 70% ethanol when changing phenotype or when the field of view would contain debris (roughly every three egg clutches). The dissected eggs were then pooled per phenotype and brought to the sterile biosafety cabinet for sterilization. Transplant 1 involved the dissection and sterilization of 235 eggs from resistant individuals and 190 eggs from sensitive individuals, while transplant 2 involved 331 eggs from sensitive individuals and 309 eggs from resistant individuals in an attempt to increase surviving snails.

Step 2: Transplant 1 followed the sterilization setup of Schols *et al.* (2023) whereby eggs were placed in the well containing bleach, the plate swirled for 10 seconds, and then the egg was moved through three washing steps involving sterilized borehole water each time moving to the next well (hence each well only comes into contact with one egg). Snail eggs were sterilized by exposure for 2min 45sec to 0.036% active chlorine concentration (LaCroix). In contrast, due to the higher egg count in transplant 2, individual egg-bleaching was no longer feasible. Therefore, eggs were placed per phenotype in 45 mL culture tubes, into which 6.93 ml of sterile borehole water was added. To this, 70 µL of undiluted bleach (3.6%) was added. This achieved the desired concentration of 0.036% in the tube. The eggs were left in these tubes for 2 minutes and 30 seconds to reduce mortality (15 seconds less exposure). At the end of this time, the tubes were filled up to 45 mL with sterile water to quickly dilute the bleach solution. New sterile borehole water was added, and the tube was then turned over 15 times to wash the eggs. This washing step was repeated two more times before the eggs were isolated and placed in individual wells.

Step 3: Microbiome donor inocula were obtained by crushing laboratory-reared snails from each phenotype in 5 mL sterile tubes with a sterilized pestle. Each inoculum was prepared at a ratio of 100 mg of snail/1 mL of sterilized water (w/v) as in Alba *et al.* (2015b) while ensuring sufficient volume for all wells. Once prepared, the inocula were kept on ice while aspirating 20µl of the resulting donor inocula to each well per treatment within 48 hours after sterilization. The remainder of the donor inocula were stored at -80°C. 48 hours after the transplant or 48 hours after hatching, whichever comes last, determined when the snail received autoclaved algae.

Step 4: One week after the first inoculum, a second inoculum was created and given to the recipients as a booster after the water of each well had been replaced with new sterile borehole water. These microbiome donor inocula were obtained as in step 3. For transplant two, these booster inocula were applied three days after the first inocula and into the petri dishes rather than the individual wells of the 24-well plate, as snails had to be moved to glass petri dishes to ensure survival. The inocula volume was scaled to the number of snails in a plate.

Step 5: 29 days after the booster microbiome (step 4), transplanted snails from each experimental group as well as control groups (untreated snails of both phenotypes) were individually exposed to five *F. hepatica* miracidia. Briefly, eggs laid by *F. hepatica* adult parasites collected from a single infected cow (Pyrénées strain, France) at the local abattoir at Perpignan were embryonated at 26°C, in the dark, for 15 days. Miracidia hatching was induced by exposing the eggs to light and experimental

infections of snails were carried out in 96-well plates (see Vázquez *et al.*, 2014 and Alba *et al.*, 2018 for details).

Step 6: At day 25 post-exposure (p.e.), shells were cleaned with cotton swabs soaked in ethanol and were then relaxed in 70°C-heated water for 20 sec to easily isolate the soft tissue with the aid of soft forceps. Each snail was snap-frozen individually in liquid nitrogen and stored at -80°C until DNA extraction. DNA from individual snail specimens was extracted, *F. hepatica* infection success was determined through diagnostic PCR (Alba *et al.*, 2015a), and the V3-V4 region of the 16S rRNA gene was amplified to study the bacterial aspect of the microbiome. Sequencing data was processed using QIIME2 and R. Quality filtering, chimera removal, and taxonomic assignment were performed, and low-abundance and contaminant ASVs were excluded. Statistical analyses included mixed-effect models for OD measurements, Kaplan–Meier survival analysis, and generalized linear models to assess infection probability and alpha diversity. Beta diversity was explored using Bray–Curtis, Jaccard, and UniFrac distances, visualized via NMDS and RDA. Differential abundance analysis was conducted using DESeq2, and clustering patterns were further examined to identify microbiome shifts associated with genotype, treatment, and infection status.

General rearing conditions: Throughout the experiment, autoclaved borehole water was used and 24-well plates and petri dishes holding snails were only opened in a sterile biosafety cabinet to avoid environmental contamination. The survival of individuals was recorded daily or every other day for as long as snail specimens were individually kept in 24-well plates. Once specimens were moved to glass petri dishes survival was only noted at milestone points as mentioned in Figure 2. Survival was determined by looking for movement or an observable heartbeat under a binocular microscope, which would correspond to surviving snails. Snails from transplant 1 were kept in individual wells in 24-wel plates until 22 days post booster transplant, being fed only autoclaved algae, after which they were moved to glass petri dishes and fed normal untreated algae. Due to the high mortality in both transplant experiments, samples of transplant 2 were moved to glass petri dishes and fed normal untreated algae three days after the first transplant had been administered.

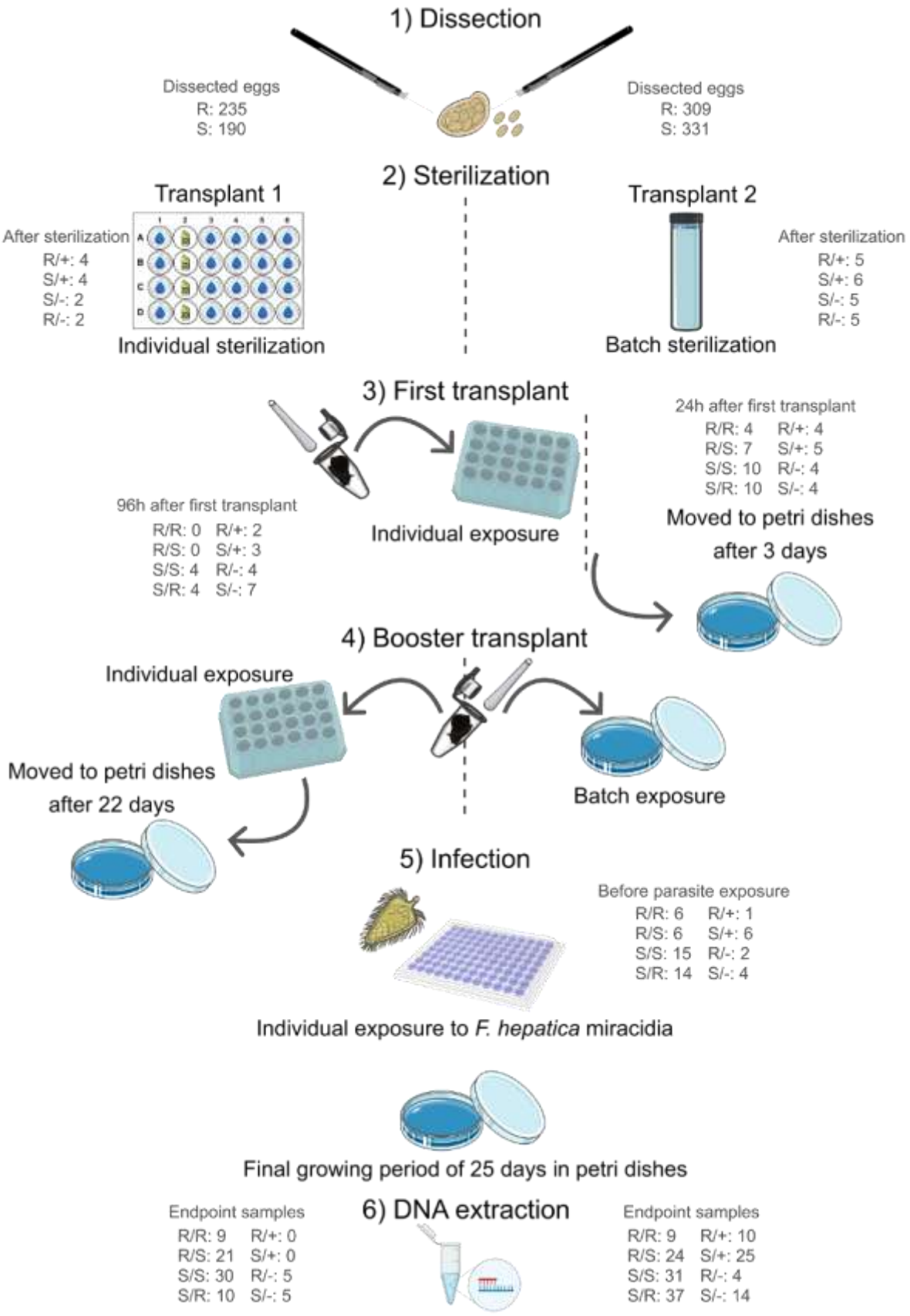

Figure 8: The six key milestones during the reciprocal transplant experiment with parasite exposure. Dotted lines indicate differences in the manipulations of transplant 1 and transplant 2 as explained in the text. The light grey text indicates the final number of samples in the microbiome dataset taken at each time point. Sample counts

on the left of the axis indicate samples from transplant 1, samples on the right from transplant 2. **1) Dissection**: dissecting *P. columella* egg masses to isolate individual eggs. **2) Sterilization**: eliminating the external native microbiota of the snail eggs through bleach exposure. Transplant 1 followed the individual sterilization setup of Schols *et al.* (2023). Transplant 2 followed a batch bleaching procedure whereby all eggs of the same genotype were bleached simultaneously. **3) First transplant**: transplanting a donor microbiome. After the first transplant, snails of transplant 1 remained in their respective well of the 24-well plate, while snails of transplant 2 were pooled in batches in petri dishes after three days. **4) Booster transplant**: transplanting a booster donor microbiome. The booster transplant was done identically to the first transplant for transplant 1, while the batches of transplant 2 were exposed in their respective petri dish. Snails of transplant 1 were moved to petri dishes 22 days after this booster transplant. **5) Infection**: 29 days after the booster transplant specimens were experimental infected with miracidia of *F. hepatica*. Each snail was individually put in a well of a 96-well plate and exposed to 5 miracidia. **6) DNA extraction**: DNA extraction followed by diagnostic PCR for infection detection and 16S rRNA amplification to study the bacterial component of the microbiome.

Aim 4 – Parasite spillback and hippo trematodes

Finally, the project examined the impact of anthropogenic pressures on snail-parasite interactions in a man-made lake in Zimbabwe. Snails were collected from the reservoir, and adult trematodes were obtained from a subadult male *Hippopotamus amphibius* culled by rangers as part of the wildlife governance quota set for population control, problem animal management, community benefits or other aspects of sustainable utilization by Zimbabwe Parks and Wildlife Management Authority. The hippopotamus was culled near Kariba Town (**Figure 9**) and the liver, bile ducts and stomach were dissected and inspected for adult trematode parasites. The collected parasites were digitized and used for morphometric measurements. Infection prevalence in snail samples was determined using classical shedding experiments and RD-PCRs (Carolus *et al.*, 2019; Schols *et al.*, 2019), and genetic analyses were conducted on both snails and parasites. This work linked trematode infections in invasive freshwater snails to infections in a keystone herbivore, the common hippopotamus, focusing on the liver parasite *Fasciola nyanzae*, and highlighted the potential threat to wildlife conservation. The study was conducted in collaboration with the University of Zimbabwe and the Kariba research station.

Attempts were made to detect *F. nyanzae* and other trematode parasites from stool samples of various large herbivores in the region (elephant, buffalo, zebra, hippo,...) to provide a non-invasive method of monitoring. However, given the highly complex nature of stool samples, the resilience of trematode eggs to DNA extraction, the low egg/stool biomass ratio, and the limited amount of time of the project dedicated to this aspect of the research, we were unsuccessful at obtaining a successful protocol.

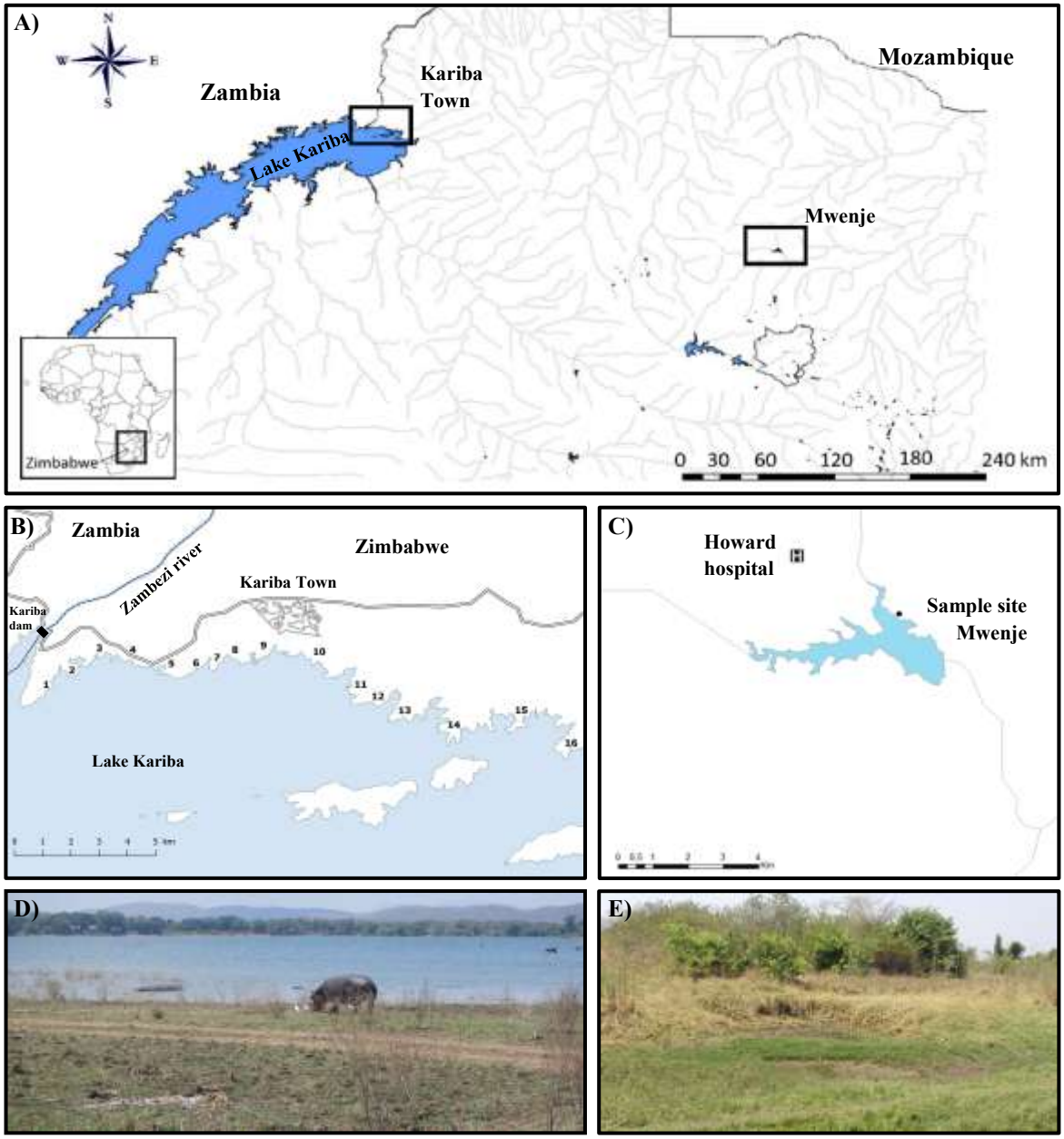

Figure 9: **A**) Map indicating the sampling locations in the Zambezi basin including 'Kariba Town' at Lake Kariba and 'Mwenje' at Mwenje reservoir in Zimbabwe. **B**) Sampling sites at Lake Kariba; figure adapted from Carolus *et al.* (2019). **C**) Sampling site at Mwenje, a temporary puddle adjacent to the main reservoir located at S 17°14'47.9" E 31°01'07.7"; figure adapted from Schols *et al.* (2020). **D**) Sampling site 13 at Lake Kariba. **E**) Sampling site at Mwenje reservoir.

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

The temporal pattern throughout schistosome development

Forty days following parasite exposure, infected and uninfected snails were compared to study the effect of parasite development on snail microbiome. When investigating the interactive effect of daysPE and infection status on the Shannon and Faith's phylogenetic diversity metrics, normality was achieved after removing the same outlier for each metric (N=1, uninfected sample at 30 daysPE). No significant effect of daysPE (Shannon: LR Chi^2= 5.38, p-value=0.37; Faith's PD: LR Chi^2=3.83, p-value=0.57), infection status (Shannon: LR Chi^2= 0.30, p-value=0.59; Faith's PD: LR Chi^2=0.87, p-

value=0.35), or their interaction (Shannon: LR Chi²= 7.43, p-value=0.19; Faith's PD: LR Chi²=8.12, p-value=0.15) was detected. However, pairwise comparisons revealed significantly higher alpha diversity (Shannon: estimate=1.24, SE=0.47, df=43, t-ratio=2.63, p-value=0.01; Faith's PD: estimate=10.23, SE=4.13, df=43, t-ratio=2.48, p-value=0.02) in uninfected samples compared to infected samples at 30 daysPE (**Figure 10A**). Shannon diversity and Faith's PD were not affected by the proportion of reads attributed to SmBRE across the entire experiment, with the exception of 30 daysPE (Shannon: LR Chi²=7.23, p-value=0.007, McFadden R^2=0.26; Faith's PD: LR Chi²=8.51, p-value=0.003, McFadden R^2=0.12). Notably, not removing the outlier with a Bonferroni p below 0.05, causes the difference between uninfected and infected snails at 30 daysPE to become non-significant (Shannon p-value=0.19, Faiths PD p-value=0.24). Bacterial load, measured via qPCR, was significantly affected by daysPE (LR Chi²= 26.24, p-value<0.001) and infection status (LR Chi²= 8.39, p-value=0.004) but not their interaction (LR Chi²= 10.39, p-value=0.06). Specifically, it was lower in infected compared to uninfected samples at 16 daysPE (estimate=0.67, SE=0.23, df=44, t-ratio=2.98, p-value=0.005).

Ordistep model selection for RDA indicated that infection status did not affect the bacterial community (F=1.03, p-value=0.40), while daysPE did have a significant effect (**Figure 10B**; F=6.01, p-value < 0.001) when considering the Bray–Curtis dissimilarity. Weighted and unweighted Unifrac distances showed an identical pattern. The within-group Bray–Curtis dissimilarity differed significantly between infected and uninfected snails at six and ten daysPE, respectively (**Figure 10C**). The core microbiome at ASV-level across uninfected samples contained an *Uliginosibacterium* sp. and a Comamonadaceae sp. at a detection threshold of 0.001 (min= 0.003, max.=0.176, mean=0.062, sd=0.047). The core microbiome at ASV-level across infected samples consisted of eight ASVs: one *Blastopirellula* spp., two *Uliginosibacterium* sp., a *Cloacibacterium* sp., a *Mycoplasma* sp., a Phycisphaeraceae sp., a Isosphaeraceae sp., and a Comamonadaceae sp. at a detection threshold of 0.001 (min= 0.026, max.=0.634, mean=0.294, sd=0.184). At 30 daysPE ten ASVs were present in all uninfected samples: two *Mycoplasma* spp., three Comamonadaceae spp., a *Chryseobacterium* sp., a *Pseudomonas* sp., a Chlamydiales sp., a Phycisphaeraceae sp., and a Caldilineaceae sp. (min= 0.068, max.=0.288, mean=0.182, sd=0.080). In contrast, 16 ASVs were present in all infected samples: two *Blastopirellula* spp., an *Uliginosibacterium* sp., a *Cloacibacterium* sp., two *Mycoplasma* spp., a *Fimbriiglobus* sp., a Bacilli sp., a Phycisphaeraceae sp., two *Pirellula* sp., an Isosphaeraceae sp., and four Comamonadaceae sp. (min= 0.224, max.=0.712, mean=0.477, sd=0.183). At the genus level *Cerasiococcus* and *Hypomicrobium* were found more abundantly in uninfected compared to infected samples (Log2fold change of 2.74 and 1.86, resp.; Padj<0.05). Further investigation of this pattern through a heatmap at ASV level did not reveal a clear pattern across daysPE.

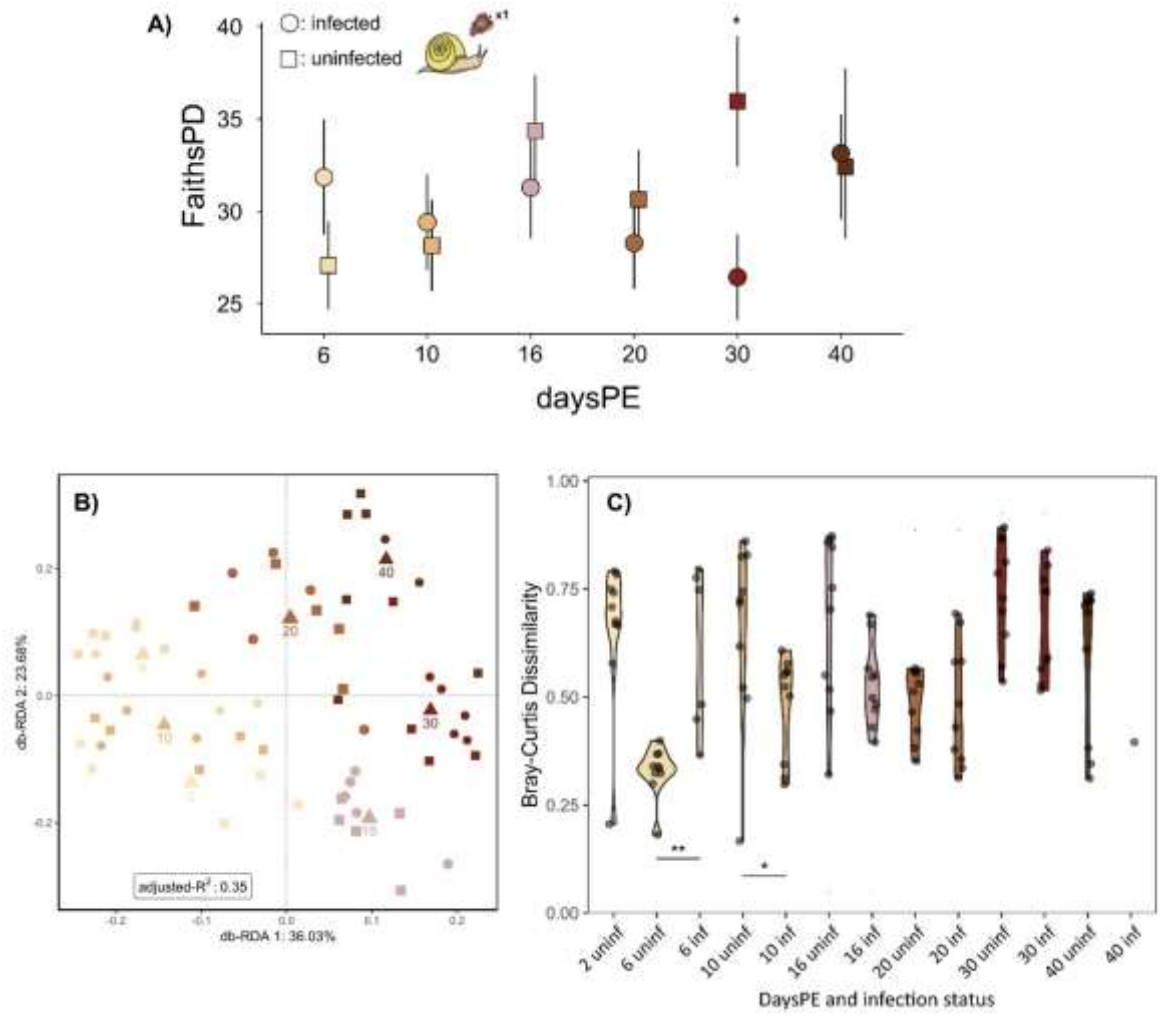

Figure 10: The alpha and beta diversity of infected vs uninfected samples across various days post SmBRE miracidium exposure (daysPE). A) Faith's phylogenetic diversity metric (FaithsPD), based on mean values with error bars representing a single standard deviation. B) Bray-Curtis dissimilarity measure as shown by RDA plot of the bacterial community adjusted for the different days post exposure. Infection status was selected out of the RDA by ordistep but is indicated for illustration purposes. The triangles are the centroids of all samples belonging to the specific post exposure day. C) Violin plots showing the within-group Bray–Curtis dissimilarity with labels on the x-axis indicating the days post exposure (first number) followed by infection status (uninf = uninfected, inf = infected). All samples at 2 daysPE are classified as uninfected since the infection status cannot be reliably determined this soon after exposure. Circles indicate infected samples, squares uninfected samples. Significance: <0.05='*' and <0.01='**'.

The effect of parasite population and species in single infections on the microbiome

To understand how different parasite species and populations affect the snail microbiome, snails exposed to three different parasites were studied 40 days after parasite exposure. For the alpha diversity, the Shannon diversity metric required inverse transformation to meet normality. Only parasite infection (uninfected, or established infections of SmLE, SmBRE and Sr) was included through stepwise model selection. The infection status significantly influenced the inverse Shannon diversity after correcting for heteroscedasticity and removing one outlier SmBRE infected sample (F= 9.29, p-value<0.001). A pairwise comparison revealed a significantly lower inverse Shannon diversity of snails infected by SmLE or Sr when compared to SmBRE (**Figure 11A**; estimate=-0.16, SE=0.05, z-value=-2.96, p-value=0.02; estimate=-0.16, SE=0.05, z-value=-3.22, p-value=0.006, resp.) and compared to

uninfected snails (estimate=-0.13, SE=0.04, z-value=-3.48, p-value=0.003; estimate=-0.13, SE=0.03, z-value=-4.14, p-value<0.001, resp.). Generally similar patterns were also noted for the inverse Faith's phylogenetic diversity metric after removing the same outlier. Stepwise selection also only kept the parasite infection in the model (LR Chi^2= 23.34, p-value<0.001). Only SmLE-infected snails remained with a significantly lower inverse transformed Faith's phylogenetic diversity from SmBRE-infected snails (estimate=0.99, SE=0.21, df=65, t-ratio=4.81, p-value=0.0001). Bacterial load, measured via qPCR, was not affected by parasite infection status (LR Chi^2= 4.07, p-value=0.25).

Ordistep model selection for beta diversity selected the additive effect of infection status (infected or uninfected) and parasite exposure (exposure to SmLE, SmBRE or Sr) when considering the Bray–Curtis dissimilarity. The entire model was significant (**Figure 11B**; F=9.55, p-value < 0.001) as well as infection status (F=3.24, p-value=0.007) and parasite exposure (F=12.24, p-value < 0.001). The same pattern is seen for the weighted and unweighted Unifrac distance, except for a significant effect of the interaction term in the former (F=2.16, p-value=0.027). The within-group Bray–Curtis dissimilarity differed significantly between all parasite infections and when compared to uninfected samples, except for snails infected with *S. rodhaini* which were not significantly differing from uninfected snails and SmLE-infected snails compared to SmBRE-infected snails (**Figure 11C**). The core microbiome at ASV-level across all samples consisted of five ASVs: a Phycisphaeraceae sp., a Planctomycetes sp., a *Uliginosibacterium* sp., a *Cloacibacterium* sp., and a Comamonadaceae sp. (min=0.019, max.=0.422, mean=0.246, sd=0.099). An additional 52 ASVs made up the core microbiome of uninfected samples (min=0.647, max.=0.783, mean=0.733, sd=0.041). When looking at the core microbiome of various infections (Sr, SmBRE, and SmLE) 21, 15, and 7 ASVs were respectively detected (resp., min=0.478, max.=0.724, mean=0.632, sd=0.073; min=0.036, max.=0.614, mean=0.401, sd=0.154; min=0.147, max.=0.822, mean=0.441, sd=0.145).

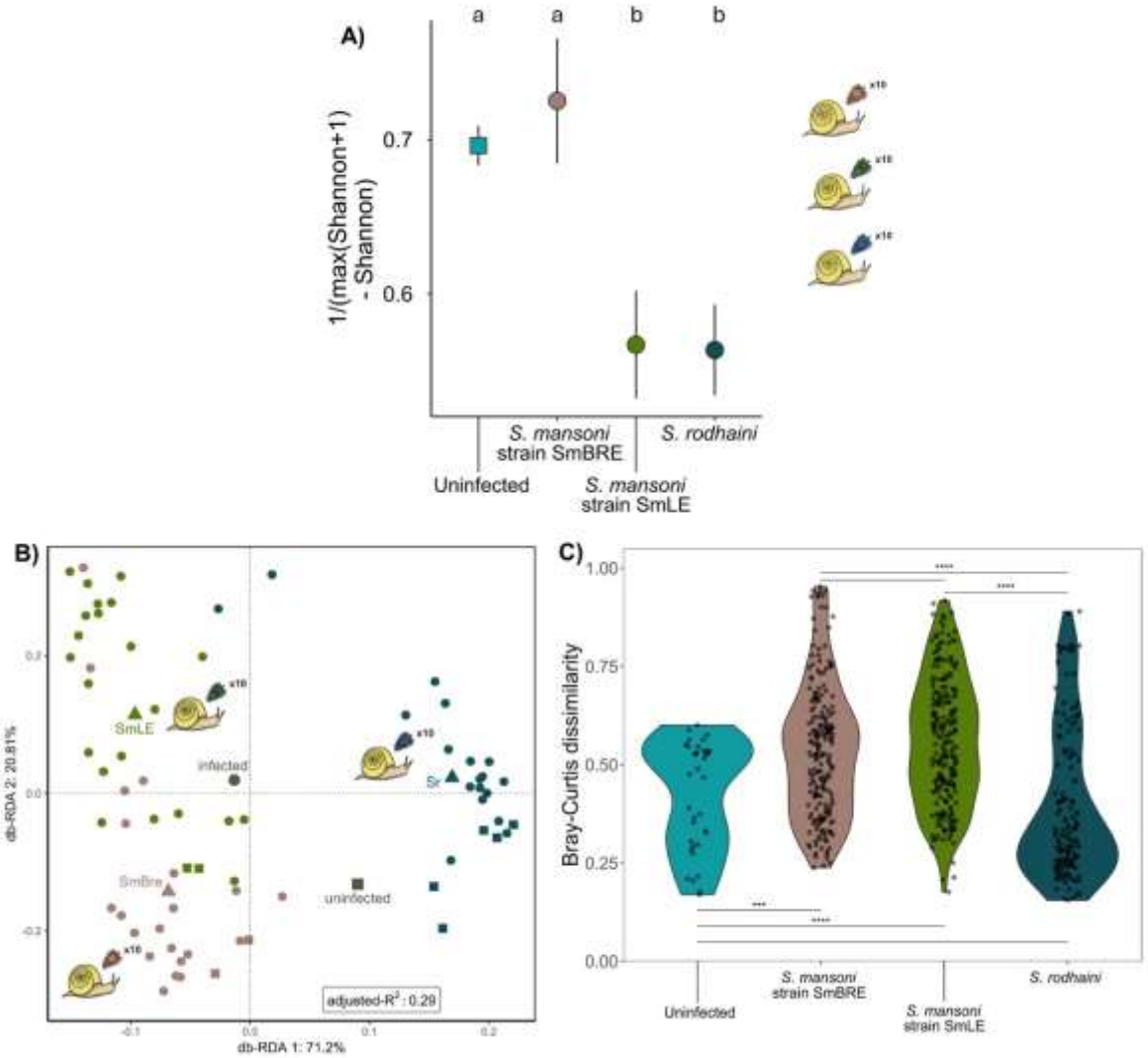

Figure 11: The bacterial aspect of the microbiome for snails 40 days post exposure to *S. mansoni* (SmBRE, low shedder), *S. mansoni* (SmLE, high shedder), and *S. rodhaini* (Sr). Uninfected samples from all exposure experiments are combined under "Uninfected" as selected for by the model. A) Inverse transformed Shannon diversity metric (alpha diversity) for snails 40 days post exposure to SmBRE (*S. mansoni* low shedder), SmLE (*S. mansoni* high shedder), and Sr (*S. rodhaini*). Significant differences are indicated by letter groups. B) Bray-Curtis dissimilarity measure of the bacterial community across the different parasite exposures as shown by RDA plot. The RDA considers parasite exposure and infection status additively. Ordistep excluded the interaction term. The triangles indicate centroid values for each parasite exposure, the large grey circle the centroid for all infected samples, and the large grey square the centroid for all uninfected samples. C) The within-group Bray–Curtis dissimilarity for the different diagnosed infections. Circles indicate infected samples, squares uninfected samples. Significance: <0.05='*', <0.01='**', <0.0001='****'.

The effect of parasite population and species under co-infection on the microbiome

Snails of two co-exposure conditions were compared during infection development to understand how co-infections shape the snail microbiome. The additive effect of daysPE (2, 10, and 40), parasite exposure (co-exposure to either SmLE and SmBRE or SmLE and Sr) and their interaction were selected by the model when considering the Shannon diversity measure. No significant effect of daysPE (LR Chi^2= 1.10, p-value=0.58) and parasite exposure (LR Chi^2= 2.02, p-value=0.15) was detected yet their interaction was significant (LR Chi^2= 10.04, p-value=0.007). Pairwise comparisons revealed a

significantly higher Shannon diversity of snails co-exposed to Sr and SmLE compared to snails co-exposed to SmBRE and SmLE at 40 daysPE (estimate=0.168, SE=0.076, df=55, t-ratio=2.19, p-value=0.03). No significant change occurred throughout the experiment in snails co-exposed to Sr and SmLE while the microbiome changed significantly when comparing ten and 40 days post-exposure (estimate=-0.18, SE=0.065, df=55, t-ratio=-2.80, p-value=0.02). A similar pattern is noted for the Faith's phylogenetic diversity metric. Also, here the interaction between daysPE and parasite exposure was significant (LR Chi^2= 11.80, p-value=0.003) while the individual variables were not. Pairwise comparisons revealed a significantly higher diversity in snails co-exposed to Sr and SmLE compared to SmBRE and SmLE at 10 daysPE (**Figure 12A**; estimate=-0.227, SE=0.082, df=55, t-ratio=-2.78, p-value=0.008) while the opposite is true at 40 daysPE (**Figure 12A**; estimate=0.164, SE=0.079, df=55, t-ratio=2.059, p-value=0.04). No significant change occurred throughout the experiment in either co-infection experiment except for snails co-exposed to SmBRE and SmLE between ten and 40 daysPE (**Figure 12A**; estimate=-0.32, SE=0.07, df=55, t-ratio=-4.738, p-value < 0.001).

RDA indicated that daysPE (F=10.88, p-value < 0.001), parasite exposure (F=4.41, p-value < 0.001) and their interaction (F=4.28, p-value < 0.001) significantly affected the bacterial community (**Figure 12B**). The same pattern is seen for the weighted and unweighted Unifrac distance. The within-group Bray–Curtis dissimilarity differed significantly between two and ten, and ten and 40 daysPE for each of the parasite exposures separately but only differed significantly at 40 daysPE between the two experimental conditions (**Figure 12C**). This pattern was not correlated with established infections. The core microbiome at ASV-level across both co-infection experiments consisted of a *Cloacibacterium* sp. and a Comamonadaceae sp. at a detection threshold of 0.001 (min= 0.002, max=0.749, mean=0.168, sd=0.145). The SmLE/SmBRE co-infection experiment core microbiome is further supplemented with an *Uliginosibacterium* sp. and an *Aquabacterium* sp. (min= 0.012, max=0.559, mean=0.205, sd=0.145) while that of the Sr/SmLE co-infection experiment was supplemented by a Planctomycetes sp. (min= 0.005, max=0.750, mean=0.200, sd=0.174). Following DeSeq2 analysis, heatmap plotting indicates stronger clustering per experiment for ten and 40 daysPE compared to two daysPE. Moreover, SmBRE and SmLE co-exposed snails showed increased read abundance of the genera *Achromobacter*, *Stenotrophomonas*, *Pedospaeraceae*, and *Thermomonas*. In contrast, Sr and SmLE co-exposed snails showed increased read abundance of the genus *Schlesneria*, and to a lesser extent *Mycobacterium* sp.

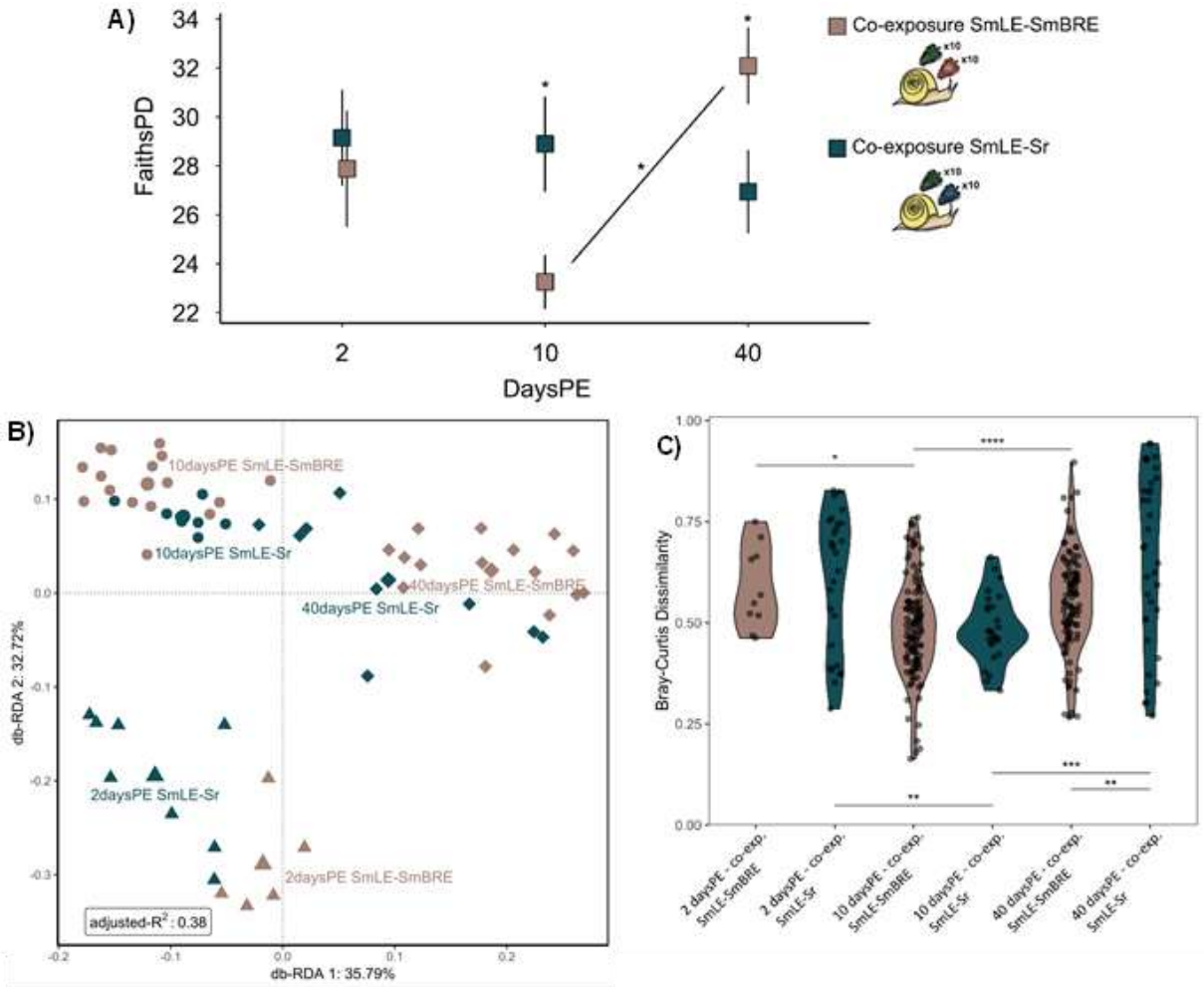

Figure 12: The bacterial aspect of the microbiome during the two co-infection experiments (SmLE & SmBRE, and SmLE & Sr; as indicated by the color code) across 2, 10, and 40 daysPE (irrespective of final infection outcome). A) The alpha diversity as calculated through the faith's phylogenetic diversity metric, based on mean values with error bars representing a single standard deviation. The line represents the pairwise comparison between 10 and 40 daysPE for the co-exposure of SmLE-SmBRE. B) Bray-Curtis dissimilarity measure across the different days post exposure (indicated by the different shapes with the centroid indicated by the largest symbol) as shown by a RDA plot. The RDA considers the co-infection experiment, days post exposure and their interaction. Ordistep excluded the infection diagnostic. C) The within-group Bray–Curtis dissimilarity with labels on the x-axis indicating the days post exposure (first number) followed by co-infection experiment ('co-exp. SmLE-Sr' and 'co-exp. SmLE-SmBRE'). Significance: <0.05='*', <0.01='**', <0.001='***', <0.0001='****'.

The first successful transplantation of the bacterial aspect of the microbiome

The donor inoculum affected overall survival chance of juvenile snails (**Fig. 10**, χ^2 (4, n = 280) = 26, p<0.001). Moreover, microbiome-disturbed snails that received a *P. corneus* donor inoculum survived better than snails without inoculum or with a *S. fuscus x S. palustris* donor inoculum (χ^2 (2, n = 60) = 6.79, p=0.02). A similar trend, although not significant, is notable for snails that received a *B. glabrata* donor microbiome (χ^2 (2, n = 55) = 5, p=0.07). The combination of all these results corroborate our hypothesis and the phylosymbiosis pattern reported by Huot *et al.* (2020), more in particular that exposure to a donor inoculum from a phylogenetically more distantly related snail reduces the fitness of microbiome-disturbed snails as opposed to exposure to a donor inoculum from a conspecific or a phylogenetically more closely related snail. Interestingly, survivorship was not significantly different when microbiome-disturbed snails were exposed to a donor inoculum originating from a conspecific

or a species from the same family (Planorbidae). A pattern possibly explained by the wild origin of the latter species as species kept in laboratory conditions tend to rapidly loose microbial diversity (Voulgari-Kokota *et al.*, 2022; Baldassarre *et al.*, 2023), counteracting the phylogenetic distance between the host species. However, since the alpha diversity measures between both donor types was not significantly different this seems unlikely (p = 1).

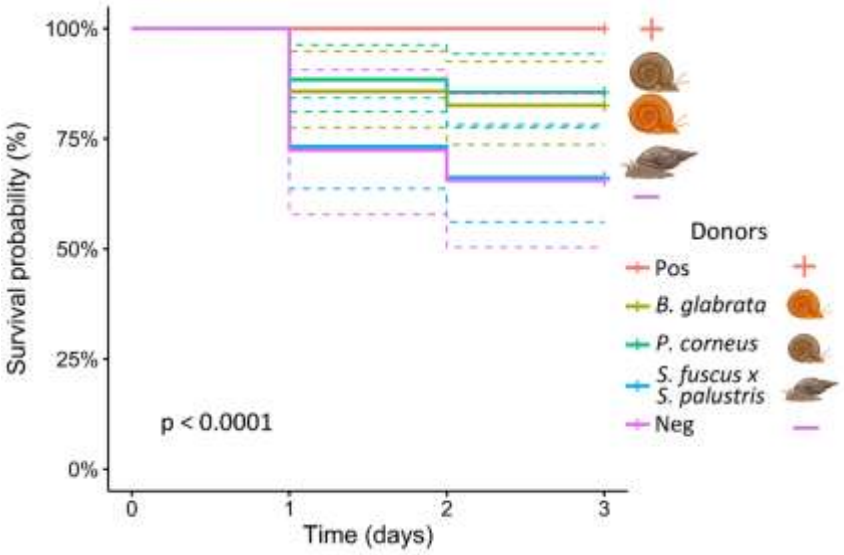

Figure 10: Kaplan-Meier survival curve across all days per donor type, excluding specimens dead at the start of the experiment or unhatched at the end of the experiment. 'Pos' refers to positive controls (untreated samples), and 'Neg' refers to negative controls (bleach-treated samples without donor inoculum exposure). Dotted lines indicate 95% CI for each respective treatment. The donor inocula were isolated from *Biomphalaria glabrata*, *Planorbarius corneus*, and *Stagnicola fuscus x S. palustris* individuals. The difference in survival probability between the different donor treatments was highly significant (p<0.0001).

The type of donor inoculum affected the bacterial community composition of the recipients (**Fig. 11**, p<0.005). Contrary to expectations, **Fig. 11a** shows each treatment type to cluster most closely to the bacterial profile of its donor inoculum yet more distant from the centroid. The previously reported phylosymbiosis pattern would suggest that a snail species assimilates more similar communities from different microbial pools, hence recipient samples were expected to be closer to the centroid. This can potentially be explained by 1) the 'Anna Karenina principle', whereby dysbiotic communities tend to be more dissimilar than healthy communities, 2) alternative stable states of bacterial communities or 3) the checkerboard pattern, whereby certain taxa tend to exclude each other from the same habitat (Levy and Borenstein, 2014; Zaneveld *et al.*, 2017). Our data seems to indicate that the former hypothesis is least probable, because individuals that received the same bacterial inoculum display lower dissimilarities compared to controls (**Fig. 11b**) (Zaneveld *et al.*, 2017). As it is unclear at this point whether the different bacterial communities have different metabolic patterns, one cannot ascertain between alternative stable states or the checkerboard pattern.

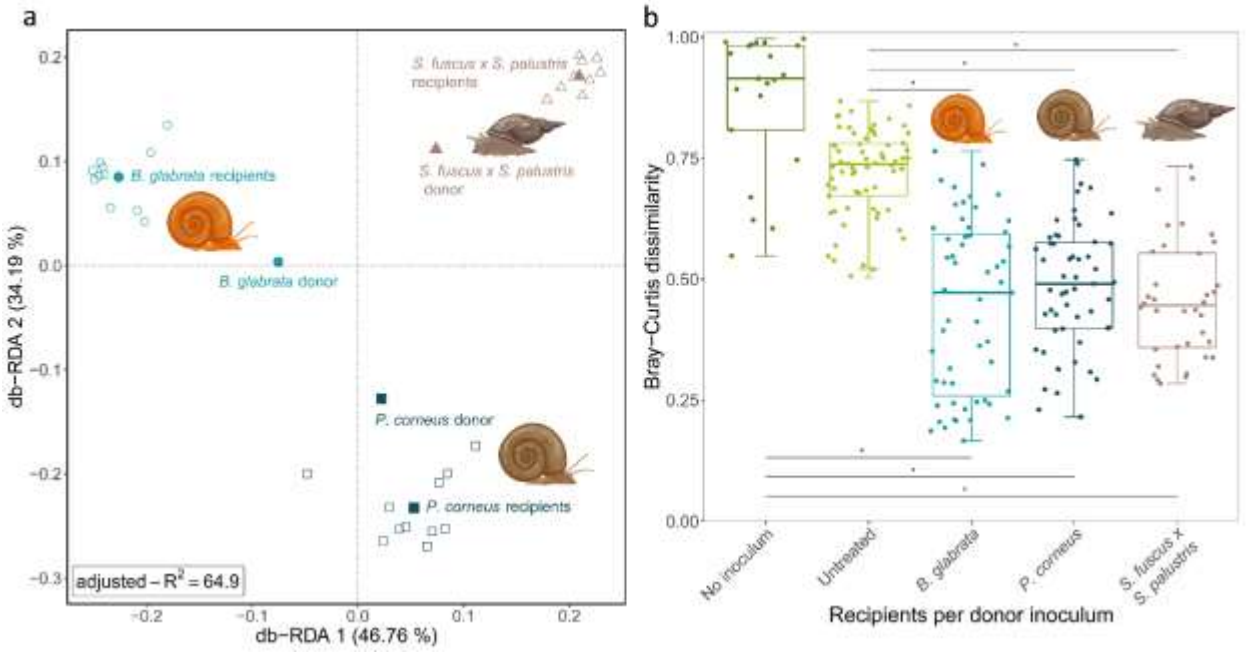

Figure 11: Beta diversity measures across the different treatments. **a** RDA plot showing the beta diversity of the samples that received a donor inoculum (Bray-Curtis dissimilarity). The model contained only the donor type and had a R^2 value of 64.9%. **b** The within-group Bray-Curtis dissimilarity (n=233). 'No inoculum' samples (negative controls) were dissected and exposed to bleach but received no donor inoculum. Untreated samples (positive controls) were dissected but were not exposed to bleach and did not receive a donor inoculum. Outliers are indicated by triangles. The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The whiskers extend from the hinge to the largest value no further than 1.5 times the interquartile range from the hinge. '*' indicates p<0.0001.

The high susceptibility of an invasive snail – what can the microbiome tell us?

Across both transplant experiments the snail's genotype purely determined the probably of infection (Chi^2= 67.53, df=1, p<0.001), there was no effect of the microbial inoculum across both transplant experiments (Transplant 1: R/R/uninfected= 9, R/S/uninfected=21, S/S/uninfected=3, S/R/infected=10 and S/S/infected=27; Transplant 2: R/R/uninfected= 9, S/R/uninfected= 3, R/S/uninfected=24, S/S/uninfected=2, S/R/infected=34 and S/S/infected=29). The mean probability of infection for resistant genotype samples was 0.03 (+-0.03) and for susceptible genotype samples 0.92 (+-0.03) whereby resistant genotype samples were never found infected.

For transplant 2: When testing the effect of genotype and control on infection status, only genotype had a significant effect (Chi^2= 25.5, df=1, p<0.001) (R/+/uninfected= 10, S/+/uninfected= 9, R/-/uninfected= 4, S/-/uninfected= 2, R/+/infected= 0, S/+/infected= 16, R/-/infected= 0, S/-/infected= 12). Bleached samples of the susceptible genotype appeared infected at lower rates; however, this was not significant (estimate = -1.06, SE = 0.80, df = 49, t-ratio = -1.32, p = 0.56). We could not assess this effect for transplant 1 as there were no bleached snails (./+) surviving until parasite exposure.

NMDS plots of the Bray-Curtis dissimilarity of transplant 2 revealed no clustering based on genotype, microbiome or infection but it did reveal separation between samples exposed to miracidia and those not exposed to miracidia (**Figure 13A**). Bray-Curtis dissimilarity, Jaccard and Unweighted Unifrac all revealed two clusters in samples exposed to miracidia. None of the variables (MiSeq run, PCR cycle nr, microbiome, genotype, infection, batch number, or their interaction) correlate with either of the two clusters. The Weighted Unifrac does not detect these two clusters nor do any of the other beta

diversity measures at taxonomic levels beyond ASV (i.e. species, genus, etc.). To investigate bacteria associated with either of the two clusters, DESEQ2 analysis was conducted. Cluster 3 and 4 were omitted in the differential abundance analysis as they only contained one and two samples, respectively. Cluster one contained 62 samples and cluster two 89, whereby 178 ASVs significantly differed between both clusters with a Benjamini-Hochberg correction for false discovery rate (**Figure 13B**). Of these, 17 ASVs were more abundant in cluster two, while the rest was more abundant in cluster one.

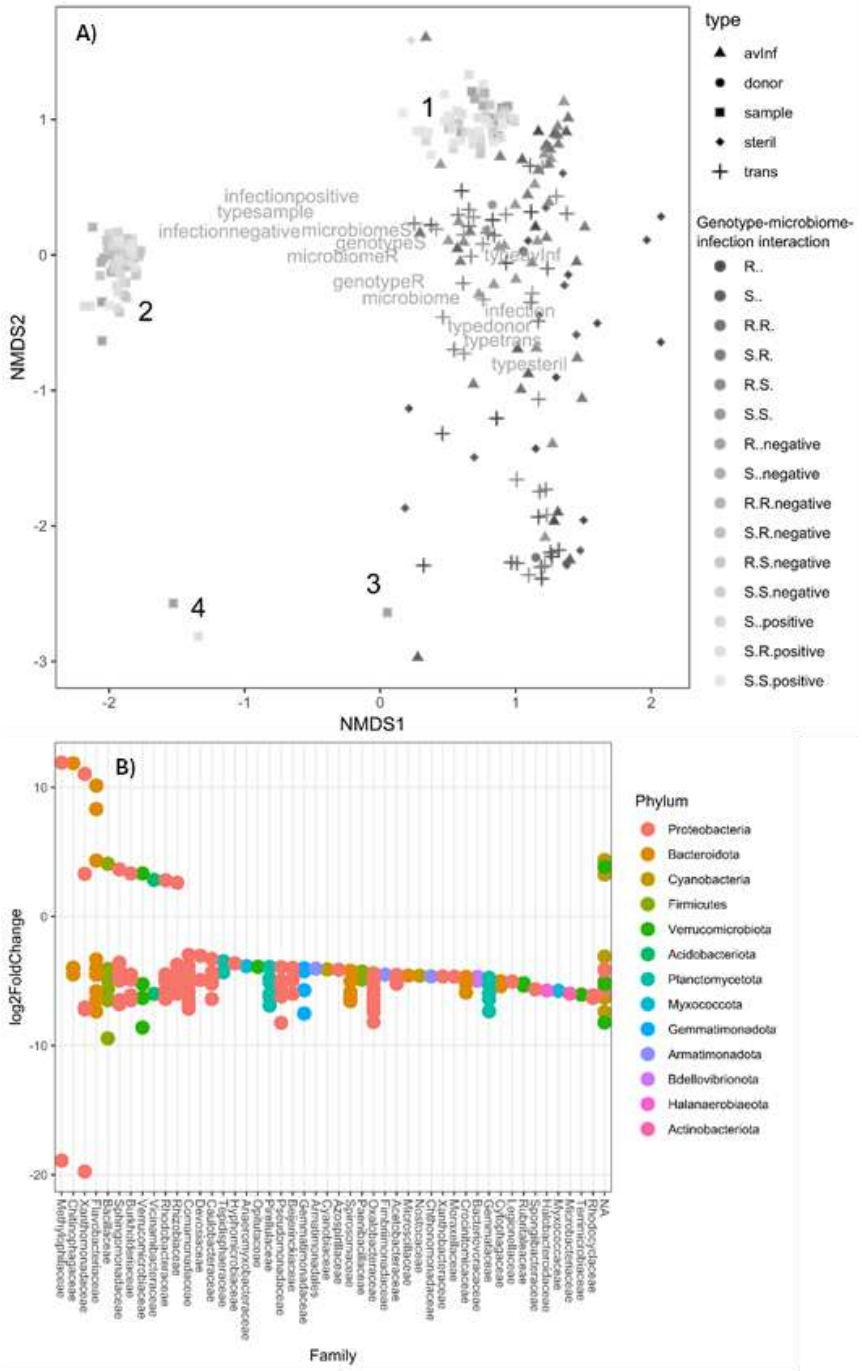

Figure 13: Beta diversity analysis of transplant 2. A) NMDS plot based on the Bray-Curtis dissimilarity with sample types indicated by shape and the genotype, microbiome and infection status interaction as color. All variables indicated by color and shape were included in the envfit analysis and fit on the NMDS ordination. Four clusters are indicated for the sample subtype (1, 2, 3 and 4), which were obtained based on an NMDS plot based on the Bray-Curtis dissimilarity focused on the sample subtype. Cluster 3 and 4 constitute only of one and two samples,

respectively, and are therefore excluded from the subsequent analyses (cluster one n=62 and cluster two n=89). B) Differential abundance analysis revealed that 178 ASVs significantly differ between cluster one and cluster two. X-axis indicates bacterial families, Y-axis the Log2 Fold change. Positive values indicate higher abundance in cluster two compared to cluster one.

For transplant 1, RDA analysis targeting donors and recipients post-transplant but pre-parasite exposure (only involving the susceptible genotype) revealed a significant difference between recipients and donors (F=6.55, p=0.02), and between recipients of either a resistant or a susceptible microbiome (F=2.94, p=0.008) of which the model combining both factors additively was significant (F=5.54, p=0.003, adj-R^2=0.53; **Figure 14A**). When looking at samples collected at the end of the experiment (**Figure 14B**), there was a significant effect of microbiome (F=3.33, p<0.001), genotype (F=3.95, p<0.001) and their interaction (F=7.41, p<0.001) on the Bray-Curtis dissimilarity model (F=5.21, p<0.001; Adj-R^2=0.15). There was no effect of infection status (F=0.99, p=0.41). The plot also reveals a separation between samples receiving a conspecific microbiome (R/R and S/S) with those receiving a heterospecific microbiome (R/S and S/R), while both heterospecific combinations also cluster separately. Samples collected in transplant 2 after the transplantation but before infection had no signal of microbiome treatment nor genotype in their microbial communities (F=0.89, p=0.67). When looking at samples collected at the end of transplant 2 (**Figure 15**), there was a significant effect of microbiome (F=5.50, p<0.001), infection (F=13.47, p<0.001) and their interaction (F=2.16, p=0.006) on the Bray-Curtis dissimilarity model (F=7.64, p<0.001; Adj-R^2=0.19). There was no effect of genotype (F=1.34, p=0.15). The plot also revealed a separation between samples never exposed to parasites and those exposed to parasites, the latter formed two separate clusters.

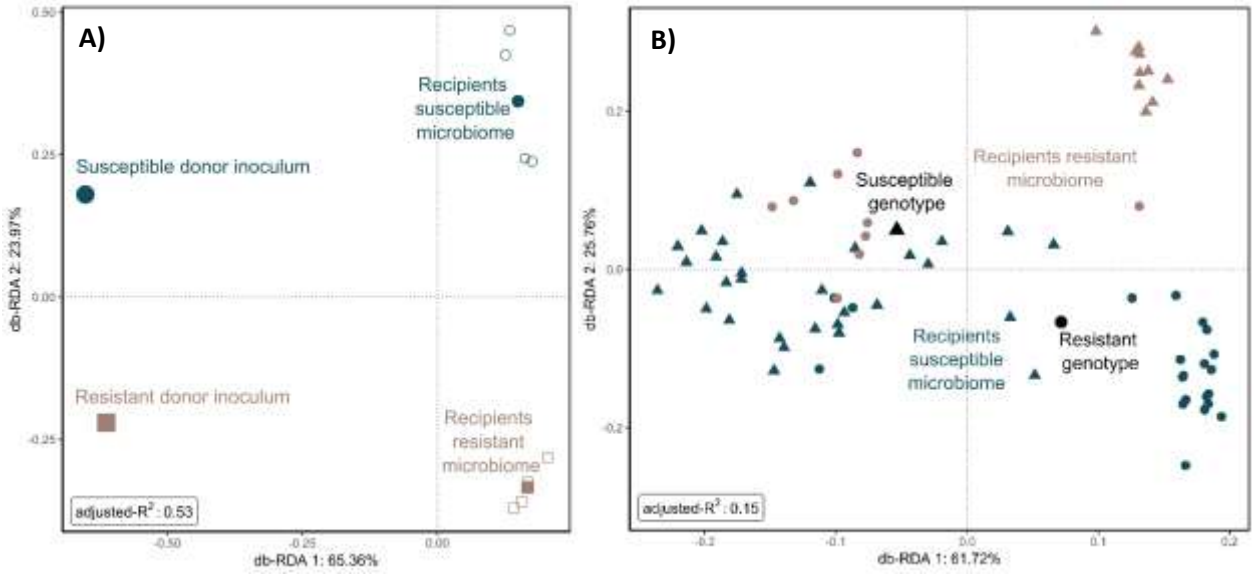

Figure 14: Beta diversity measures of transplant 1 across samples, donors, genotype and microbiome inoculum. A) Bray-Curtis dissimilarity measure of the bacterial community across the different microbial inocula for recipients and donors collected four days after inoculation as shown by RDA plot. The RDA considers the interaction of sample type and microbial inocula. B) Bray-Curtis dissimilarity measure of the bacterial community across the different recipients (microbial inoculum and recipient genotype) collected at the end of the experiment as shown by RDA plot. The RDA considers the genotype, the microbial inocula and their interaction. The circles indicate the resistant genotype, while the triangles indicate the susceptible genotype. Color represents microbial inoculum.

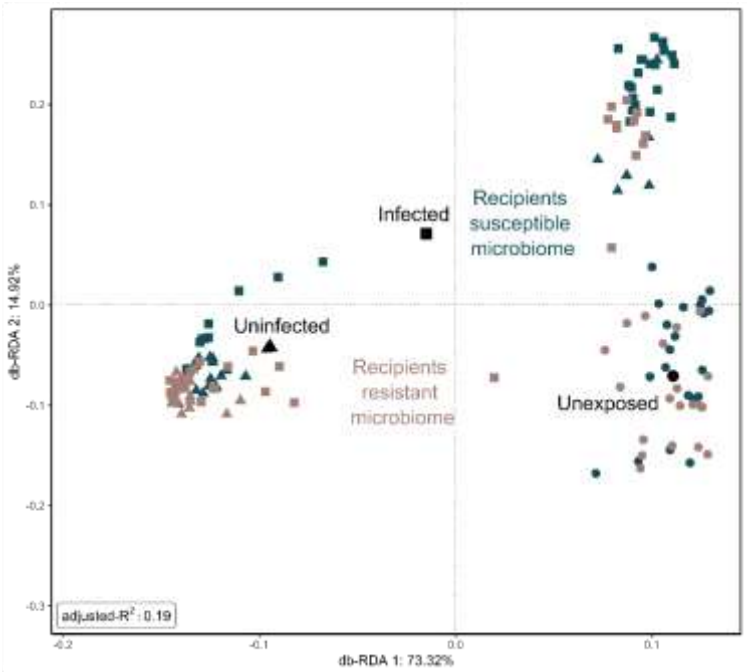

Figure 15: Beta diversity measures of transplant 2 across samples, infection status and microbiome inoculum. Bray-Curtis dissimilarity measure of the bacterial community across the different recipients (microbial inoculum and infection status) collected at the end of the experiment as shown by RDA plot. The RDA considers microbial inoculum, infection status and their interaction. Ordistep excluded genotype. The circles indicate unexposed samples, the triangles indicate uninfected samples, and the squares infected samples. Color represents microbial inoculum.

Transplant experiments go beyond correlations and reveal the presence or absence of causative links by actively or passively introducing bacterial strains or communities to recipients (Greyson-Gaito *et al.*, 2020). In doing so, earlier works have revealed that bacterial communities are at the root cause of parasite resistance in bumblebees (Koch and Schmid-Hempel, 2012) and cyanobacteria tolerance in water fleas (Macke *et al.*, 2017a). We here attempted two such transplant experiments in the globally invasive snail *P. columella* to decipher potential microbiome-related drivers of resistance to trematode parasites.

Pseudosuccinea columella can be a highly suitable host for the transmission of *F. hepatica* (Krull, 1933; Dar *et al.*, 2015; Alba *et al.*, 2019)*, F. gigantica* (Grabner *et al.*, 2014) and *F. nyanzae* (Carolus *et al.*, 2019; Schols *et al.*, 2021). However, some *P. columella* populations from Cuba are resistant to *F. hepatica* infection (Gutiérrez *et al.*, 2003; Alba *et al.*, 2018, 2020, 2022). Moreover, while susceptible *P. columella* populations in Cuba contribute less to *Fasciola* transmission than the local snail *Galba cubensis* (Vázquez *et al.*, 2014), the introduced populations in Africa play a bigger role in transmission compared to the local African species, displaying unusually high infection rates ((Carolus *et al.*, 2019; Schols *et al.*, 2021). This suggests an increased susceptibility of the invasive populations. Indeed, sympatric and allopatric combinations of *P. columella* with *F. hepatica* show highly variable compatibility patterns (Vázquez *et al.*, 2019). This could fit with earlier findings where the host-associated microbiome can shape host phenotypic traits and facilitate adaptation to novel environments (Macke *et al.*, 2017a; Henry *et al.*, 2021). On a higher level, if the host is a keystone species, the microbiome can indirectly structure environments acting as an important contribution for ecosystem engineering and eco-evolutionary interactions (Decaestecker *et al.*, 2024). Although not being described as keystone species, freshwater snails are important for aquatic ecosystems (Brönmark, 1989). Moreover, freshwater snails have been shown to acquire novel bacterial members within their invasive range (Bankers *et al.*, 2021). Combined these results suggest that *P. columella*

might assimilate a new microbial community within its invasive range, potentially facilitating its invasion and its high transmission of *Fasciola* parasites. Although our reciprocal transplant experiment (Aim 3) contained specimens from its invasive range in France, it did not contain wild-caught snail specimens, leaving this question unanswered (Alba *et al.,* in prep.). However, our reciprocal transplant experiment argues against this hypothesis since resistance could not be modulated through microbiome transplantation. Nevertheless, the hypothesis that snail adaption to the environment and parasite infection is mediated through the microbiome remains open.

Resistant snails show a consistently increased immune response compared to susceptible snails (Gutiérrez *et al.*, 2003; Alba *et al.*, 2020), yet it does not protect them from infection by echinostomes and *Trichobilharzia* sp., suggesting a parasite-specific response (Alba *et al.*, 2022). Depending on the specificity of this response, transplantations might still induce resistance to *F. gigantica* or *F. nyanzae*. Therefore, future research should first discern if resistance in *P. columella* extends to these two fasciolids. Moreover, future research should investigate if the microbiome can be linked to this increased immune response in resistant snails. Nevertheless, microbial transplants do not always depend on the upregulation of the host immune system (Chrostek *et al.*, 2014; Mazuecos *et al.*, 2023), potentially circumventing the snail's immune response to induce resistance to trematode infection. Moreover, overwhelming evidence exists for *Wolbachia*-reduced transmissibility of pathogens by insects (Moreira *et al.*, 2009; Pinto *et al.*, 2021; Shi *et al.*, 2023), yet occasionally the opposite is found (Hughes *et al.*, 2012; Dodson *et al.*, 2014; Zélé *et al.*, 2014). Moreover, this response appears to be strain-specific (Osborne *et al.*, 2009). Combined with the strain-dependence of compatibility in the snail-trematode system (Webster and Woolhouse, 1998; Lockyer *et al.*, 2012; Nacif-Pimenta *et al.*, 2012), other strain or species combinations might be amendable for microbial transplants.

If anything, the high specificity of resistance holds promise for a sustainable solution within the *One Health* framework, as it would enable the targeted elimination of human pathogens without affecting other parasite fauna and thereby ecosystem functioning. The *One Health* paradigm is especially relevant for trematodiases as they are involved in many biological interactions, across animal kingdoms and environmental conditions (Loker *et al.*, 2022). Although the removal of one parasite can alter the transmission pattern of another (Laidemitt *et al.*, 2019), it can be argued that these effects are more desirable than completely removing all trematodes, snails and other aquatic life through the use of frequently toxic molluscicides (WHO, 1965; Dai *et al.*, 2008). Even so, it will be important to obtain a targeted control effort as parasites can also protect their host from viral infections (Wuerthner *et al.*, 2017). Nevertheless, close monitoring of these systems and a profound understanding of the biology will be vital to detect and intervene at an early stage of a trematode outbreak.

The effect of trematode infection on the snail-associated microbiome

Earlier works report the effect of pathogen or parasite infections on the host-associated microbiome (Jani *et al.*, 2021; Rajarajan *et al.*, 2022). Jani *et al.* (2021) revealed that the skin bacterial profile of frogs shifted after fungal infection, while Rajarajan *et al.* (2022) showed that the gut bacteria of *Daphnia longispina* changed after infection with an eukaryotic gut parasite. Likewise, trematodes consistently drove changes in the bacterial aspect of the snail microbiome, although some more than

others and differently throughout bacterial metrics (Schols *et al.*, 2025 and Aim 3). For example, *S. mansoni* strains were more likely to drive bacterial dysbiosis compared to *S. rodhaini* in *B. glabrata,* while in co-exposure *S. rodhaini* managed to stabilize the effect of a dominant *S. mansoni* strain on the bacterial profile (Schols *et al.*, 2025b). Most importantly, we revealed that these shifts correlate with key moments in the intramolluscan development of the parasite. In our transplant experiment involving *P. columella* we hint to alternative stable states or the checkerboard pattern potentially explaining the observed clusters in our bacterial dataset. Similarly, *Batrachochytrium dendrobatidis* infection can lead to alternative stable states in the skin bacterial communities of frogs (Jani *et al.*, 2021). It appears to be a rather ubiquitous pattern as nematode infections in both terrestrial slugs and one of our used model organisms, *B. glabrata,* also show a shift in the bacterial aspect of their microbiome (Osório *et al.*, 2020). Whether this bacterial shift occurs due to the snail's immune response, the parasite multiplying and damaging host tissue, or an interplay between the snail and parasite, remains to be investigated. Future experiments could compare signals in the microbiome across artificial (Ittiprasert and Knight, 2012; Pila *et al.*, 2016) or parasite-induced (Núñez *et al.*, 1994; De Jong-Brink *et al.*, 2001) snail immune responses. These experiments supplemented by histopathological examination of infected snails, which also focus on tissue-specific microbiota, should reveal what drives the observed bacterial shifts reported throughout this project.

Indeed, bacterial shifts occur during mechanical damage and biochemical changes at the onset of shedding, and at the onset of migration to the gonad-digestive gland region (Schols *et al.*, 2025b), suggesting the importance of tissue damage in this pattern. However, Allan *et al.* (2018) linked the presence of a genomic region that confers schistosome resistance, to the presence of different bacterial species in or on snails, which suggests that the snail immune response might also be a major driver. Trematodes can modulate the snail's hemocytes to avoid destruction (Núñez *et al.*, 1994; De Jong-Brink *et al.*, 2001), which affects its capacity for bacterial clearance, providing a parasite-induced effect on the host's immunomodulation of the associated microbiome (Núñez *et al.*, 1994). Across host-parasite systems, parasites have been shown to modulate the epigenome of their parasitized host (McMaster *et al.*, 2016). Specifically, *S. mansoni* subverts the stress response of *B. glabrata* by modulating the epigenome to ensure successful infection development (Knight *et al.*, 2016; Geyer *et al.*, 2017). The microbiome is being put forward as potentially shaping a host's phenotype through epigenetic regulation by, for example, influencing the availability of chemical building blocks involved in epigenetic regulation, affecting host-associated processes directly involved in epigenetic pathways, or influencing the expression or activity of epigenetic regulatory enzymes (Chernov *et al.*, 2015; Woo and Alenghat, 2022). Combined, the potential interaction between the microbiome and the parasite-mediated epigenetic modulation of the snail showcases the importance of epigenetic studies in trematode resistance. In conclusion, all the abovementioned factors might be interacting and driving the observed patterns in the snail bacterial profiles.

Microbiome-mediated control of trematodes – a dead end?

In Schols *et al.* (2023) we showed the possibility of transplanting the bacterial aspect of the microbiome in and between freshwater snails. Indeed, from earlier works, it became apparent that it should be feasible due to the plasticity of the snail associated bacteria (Chernin, 1957, 1960; Ducklow

et al., 1981). Chernin (1957), like us, was able to obtain high proportions of germ-free snails by treating the external egg surface. This suggests that the parental snail does not, or at least not consistently, transmit bacteria vertically within the egg (Ducklow *et al.*, 1981). This conflicts with the argument of consistent vertical transmission made by Lin *et al.* (2023). Nevertheless, pseudo-vertical transmission (Alberdi *et al.*, 2016) can still play a major role in the snail microbiome as the gelatinous matrix of the egg mass contains a significant microbial community (own observations). Indeed, other studies have shown that snails contain some overlapping bacterial species between their invasive range and their native region (Bankers *et al.*, 2021). For now, it remains unknown whether the bacteria co-invaded with the snails out of the native region, favoring the importance of (pseudo-)vertical transmission, or whether these bacteria are consistently accrued form the environment (Bankers *et al.*, 2021). Either way, this reveals a readily amendable microbiome that is key for microbial transplants to work.

Temperature changes can modulate snail resistance to trematode infections (Ittiprasert and Knight, 2012) and have been shown to affect the efficiency of symbiont-mediated protection, e.g. from viral infections in an insect host (Chrostek *et al.*, 2021). Before exposure, the snail immune response can be artificially modulated by injecting a granulin growth factor, which induces hemocyte proliferation, making snails resistant to parasite infection (Pila *et al.*, 2016). During infection development, the reproductive capacity of the parasite can be impaired by introducing *Nosema algerae*, a microsporidium isolated from mosquitoes, which infects the parasite sporocysts and effectively reduce the eventual shedding load of *B. glabrata* (Lai and Canning, 1980). These examples underline the possibility of bioengineering resistance (Le Clec'h *et al.*, 2022; Sun *et al.*, 2024). However, transplanting the microbiome from resistant snails to susceptible snails does not confer resistance to trematode infection in some populations of *P. columella*. Supporting this finding, an ichtyosporidean pathogenic to *Schistosoma mansoni* occurred more in resistant than susceptible snails yet was not involved in conferring that resistance (Hertel *et al.*, 2004). These results might be interpreted as a dead end for microbiome-mediated trematode control. However, some host phenotypes are not driven by the microbiome nor do all microbes shape host phenotypes (Henry *et al.*, 2021). Moreover, host-microbiome interactions can be genotype specific (Macke *et al.*, 2017b; Decaestecker *et al.*, 2024). This suggests that we might have excluded host genotype-microbiome combinations which would have shown a phenotypic response. A case can be made that recent works have only scratched the surface of the snail-trematode-microbiome interaction. Snails have close associations with a complex set of symbiotic bacteria, fungi, ichtyosporideans, microsporidia, protozoa, nematodes, Oligochaeta, viruses and rotifers (Fashuyi and Williams, 1977; Hertel *et al.*, 2004; Galinier *et al.*, 2017; Clerissi *et al.*, 2023), each offering avenues for snail control (Duval *et al.*, 2015) or reducing parasite load (Lai and Canning, 1980). Our work focused on an amplicon-based approach of the bacterial aspect of the complex microbiome. It paves the way for more in-depth techniques as well as various other aspects of the microbiome whereby bacteria could still be critical for the complex interactions surrounding trematode resistance.

All these potential microbiome-mediated control measures to increase snail resistance still hinge on the fact that some level of parent offspring transmission occurs, while ideally providing a fitness benefit so that it spreads naturally throughout snail populations. Given the incomplete inheritance

this might become problematic (Schols *et al.*, 2023). However, since snails even in different continents accrue similar bacteria (Bankers *et al.*, 2021), there is indeed hope that it is possible to get (pseudo-)vertical transmission when a trematode resistance-conferring microbe is found. Alternatively, snail-resistance could be achieved if resistance-inducing strains enter the environment and hosts accrue these environmental bacteria in their microbiome (Decaestecker *et al.*, 2024). This approach holds promise, since snails have a modifiable microbiome (see earlier). However, snails raised in a common garden showed a phylosymbiosis pattern with their bacterial profiles, indicating that they will not accrue just any bacterial community (Huot *et al.*, 2020). This specificity is an important factor to consider for future applications of microbiome manipulations.

More attempts are to be made on the reciprocal transplant experiment, focusing now also on pre-exposed microbiome transfers. Hosts previously exposed to pathogens have a microbiome that shows enhanced resistance to infection (Stacy *et al.*, 2021). Transplanting this microbiome to unexposed hosts subsequently increases their tolerance (Macke *et al.*, 2017a). Indeed, a significant shift is noted after parasite exposure (Schols *et al.*, 2025b), suggesting the potential of pre-exposed microbiota to confer resistance.

If eventually resistance-inducing bacteria do not exist within the snail-trematode system under study, we might still find them in other systems. As mentioned earlier, microsporidia isolated from mosquitoes can reduce parasite load in snails (Lai and Canning, 1980). Similarly, *Wolbachia* from *Drosophila melanogaster* has been introduced in *Aedes Aegypti* to reduce dengue transmission (Hoffmann *et al.*, 2011). These examples reveal the value and future interest in the inter-species exchange of microbial components.

Alternatively, the microbiome can be engineered to confer resistance (Mueller and Sachs, 2015). Bacteria can have intrinsic anti-tumor activity or stimulate the host's immune response to tumor cells, providing an important treatment option to certain cancer types (Zhou *et al.*, 2018). Bioengineering these bacteria to improve safety, increase tumor targeting, and heighten effector molecule production should improve the applicability of this therapeutic approach (Zhou *et al.*, 2018). The microbiome is intimately linked to the host immune system through the production of outer membrane vesicles, antigens and metabolites (Brestoff and Artis, 2013; Kaparakis-Liaskos and Ferrero, 2015; Rooks and Garrett, 2016). Therefore, the resistance obtained through hemocyte boosting with granulin injections (Pila *et al.*, 2016) might be achievable through microbiome manipulations. Indeed, bacterial and viral pathogens can readily modulate host immune responses (Finlay and McFadden, 2006), and endosymbionts can manipulate host gene expression affecting disease resistance (Hussain *et al.*, 2011). These examples suggest that we could apply this microbiome-modulation to stimulate immune responses as discussed for outer membrane vesicles (Kaparakis-Liaskos and Ferrero, 2015).

In contrast to the bacterial aspect of the snail microbiome, it appears that the trematode bacterial profiles have some core members that can be transmitted across and between life stages (Fischer *et al.*, 2017; Jorge *et al.*, 2020). Therefore, future research could focus on the microbiome of the disease agent itself, rather than that of the intermediate host. Indeed, microbial symbionts can be critical for pathogen survival (Taylor *et al.*, 2005), while also determining disease severity (Ives *et al.*, 2011). We

might be able to find and target a vital symbiont of trematodes, similarly to how antibiotics kill the *Wolbachia* symbiont and consequently the filarial nematode within the human body (Taylor *et al.*, 2010; Landmann *et al.*, 2011). It is promising that certain bacteria are transmitted across generations and life cycle stages (Jorge *et al.*, 2020). Moreover, antibiotic treatment changes the ratio of soldier to reproductive stages within a snail host, suggesting a potentially important role of bacteria in the lifecycle biology of trematodes (Jorge *et al.*, 2022a). However, another study found only a weak correlative link between bacteria and alternative lifecycles within a trematode species (Salloum *et al.*, 2023a). Either way, this aspect of the snail-trematode-microbiome interaction appears promising and should be further investigated.

Miracidia respond to chemical cues released in the water by compatible freshwater snail hosts while ignoring those of incompatible sympatric snail species (Kalbe *et al.*, 1997; Hertel *et al.*, 2006; Allan *et al.*, 2009). Consequently, miracidia transmission success is increased markedly (Hertel *et al.*, 2006). However, transmission frequently does not depend on miracidia failing to locate the intermediate snail host (Gutiérrez *et al.*, 2003). Nevertheless, there might be value in making decoy snails attractive or compatible snails unattractive to miracidia by manipulating the cues their release (discussed in Loker *et al.*, 2022). Perhaps microbial organisms can be applied to achieve this (Archie and Theis, 2011). Indeed, bacteria associated with caterpillars release attractant molecules, aiding their localization by hyperparasitoid wasps (Bourne *et al.*, 2023). Moreover, this attraction was successfully induced artificially (Bourne *et al.*, 2023), revealing the potential of this approach. Similarly, the human skin microbiome determines our attractiveness to malaria-transmitting mosquitoes (Verhulst *et al.*, 2011).

This reveals another target for microbiome-mediated control of trematodiases – hiding the final host. Indeed, cercariae require certain chemical compounds, in combination with other stimulants like temperature and water turbulence, to attach to their final host (Haas *et al.*, 2002). Notably, the drivers of this response can be constant across taxonomic levels yet also variable, even within genera (Haas *et al.*, 2002; Horák *et al.*, 2015). Nevertheless, it is a promising avenue since an adapted DEET formula applied to the skin has been shown to prevent cercarial penetration (Salafsky *et al.*, 1999). However, this effect is most likely induced by preventing penetration or killing cercariae on the skin (Salafsky *et al.*, 1999). Similarly, symbiotic rotifers living in close association with *B. glabrata* have been shown to produce a chemical capable of paralyzing schistosome cercariae (Gao *et al.*, 2019).

Targeting key development moments within the snail might provide an interesting alternative to resistance at the initial infection stage. Indeed, *Wolbachia* infections in mosquitoes offer similar opportunities with severely reduced transmission to the next host by preventing infection but also by restraining the formation of the infective stage, the sporozoite (Wang *et al.*, 2012; Gomes *et al.*, 2017). Tavalire *et al.* (2016) posit a similar perspective after observing delayed shedding of up to 30 weeks in certain inbred *B. glabrata* lines with equal resistance to infection. Moreover, snail lines capable of delaying the onset of shedding, and thus castration, also increased their reproductive period thereby underlining the viability of this target for fixation in natural populations (Tavalire *et al.*, 2016). Perhaps the microbiome was an underlying factor driving this delayed shedding which may be induced

artificially through microbial manipulations. To test this hypothesis and ascertain a causative relationship between the microbiome and the onset of shedding, future experiments may attempt to artificially increase the alpha diversity around the 30-day mark and study the effect on cercarial release.

Alternatively, we might not focus on making susceptible snails more resistant but on making resistant snails better competitors. Typically, resistance and competitive ability are at a trade-off (Wolinska and King, 2009), yet the microbiome can alter the mean and variation of phenotypic traits (Henry *et al.*, 2021). As a result the microbiome members and their interactions have been critical in shaping host fitness and lifespan (Gould *et al.*, 2018). As organisms age, the immune system and microbiome change composition and function, increasing susceptibility to pathogens (Bosco and Noti, 2021). Moreover, transplanting the microbiome of old mice to young ones reduces their lifespan (Lee *et al.*, 2020). The opposite is also true whereby microbial transplants can reverse symptoms of aging (Parker *et al.*, 2022; Meng *et al.*, 2023). Perhaps this can lay the basis for increased fitness avenues for resistant snail genotypes. If the microbiome eventually does not induce resistance, it might still be valuable as control efforts to target and kill invasive snails (Lin *et al.*, 2023). Nevertheless, extrapolating such interventions to the field can be complex and is not without risk (Greyson-Gaito *et al.*, 2020), warranting a deeper understanding of the snail microbiome before any field interventions are considered.

Microbiome manipulations in the field – things to consider

Critical insights have come from controlled experiments investigating the importance of the microbiome in host biology (Greyson-Gaito *et al.*, 2020). The isolation of microbiome-mediated effects from other factors, such as environment and host physiology, has been critical in eliminating potentially confounding factors and discern a true microbiome-driven effect. Consequentially, the ecological relevance of many of these experiments has remained limited (Greyson-Gaito *et al.*, 2020). Therefore, putative resistance-inducing bacteria should be tested in different ecological and host physiological contexts. However, these experiments should first occur in controlled settings as a stepping-stone to field manipulations since biological control can have unexpected impacts on an ecosystem (Howarth, 1991; Appleton *et al.*, 2009; Wong *et al.*, 2009; Ip *et al.*, 2014) and incomplete resistance may lead to increased pathogen virulence (Gandon *et al.*, 2001; Fleming-Davies *et al.*, 2018). Nevertheless, we can take inspiration and caution from, for example, aquaculture, agriculture and mosquito-borne disease control efforts once these experiments are on the radar.

The agricultural sector has been leveraging microorganisms in the field for decades by treating seeds, plants and soils with microorganisms for nitrogen fixation, killing pest insects, and increasing stress-tolerance (French *et al.*, 2021). Similarly, incorporating prebiotic or probiotic treatment of the environment or food could drive snail-microbiome associations, as it does for the microbiome of fish in aquaculture (Denev *et al.*, 2009; Foo *et al.*, 2017). Its efficacy could be augmented by customized microbiome manipulations tailored to the pond or snail population, similar to the field or crop specific microbiome management in agricultural systems (Sergaki *et al.*, 2018; French *et al.*, 2021). However, the immense amount of environmental bacteria may interfere with or dilute the desired effect of inocula (Čaušević *et al.*, 2024), suggesting that directly manipulating (pseudo-)vertically-inherited

endosymbionts of snails remains a preferred mode of action. Indeed, mosquito-borne diseases have been successfully reduced by placing buckets with manipulated eggs in the desired region (Anders *et al.*, 2018) to release endosymbiont-manipulated mosquitoes in the environment (Pinto *et al.*, 2021; Ogunlade *et al.*, 2023). The simplicity of this release method contributes to the high cost-efficiency of this control measure against mosquito-borne diseases (Zimmermann *et al.*, 2024).

How one snail can threaten the enigmatic hippopotamus

Fasciola nyanzae infections were detected in three lymnaeid snail species: the endemic *Radix natalensis,* the Asian *Radix* aff*. plicatula,* and the North American *Pseudosuccinea columella*. The latter two species are invasive non-endemic snails that could affect parasite transmission in three distinct ways: parasite spillover, parasite spillback or a dilution effect. Co-invading parasites, like other invading fauna and flora, typically go through a genetic bottleneck, resulting in low genetic diversity in the invasive population. In contrast, our *F. nyanzae* samples show a high genetic diversity with 9 out of the 11 sequenced *COI* haplotypes being unique. Moreover, their definitive host (*H. amphibius*) and at least one of their intermediate hosts (*R. natalensis*) are endemic to Zimbabwe, supporting the endemicity of *F. nyanzae* to this region. In addition, another requirement to comply with a 'parasite spillback' hypothesis was met, as a higher *F. nyanzae* infection prevalence was detected in the invasive snail *P. columella* compared to native snails. We can therefore confirm that the cascade of biological invasions, in which the invasion of *P. columella* was facilitated by the introduction and subsequent colonization of water hyacinth from South America as described in Carolus *et al.*, has led to 'parasite spillback' of the endemic parasite *F. nyanzae* in Lake Kariba. Water hyacinth and lymnaeid snails such as *P. columella* generally thrive in nutrient rich, still or slow-moving water, but not in fast moving riverine systems, like the Zambezi river, which occupied the Kariba gorge before the construction of Kariba dam. Therefore, we hypothesize that the parasite spillback phenomenon we witness here, is a result of this man-made impoundment.

Large herbivores like *Hippopotamus amphibius,* the common hippopotamus, have a disproportionately large effect on the ecosystems they inhabit (Stears *et al.*, 2018; Ripple *et al.*, 2023). In contrast, parasites can drastically alter the impact an organism has on its environment (Collinge *et al.*, 2008; Smith *et al.*, 2009; Tompkins *et al.*, 2011). Despite the importance of large herbivores and the impact of parasitic infections, the knowledge of parasites of large herbivores remains fragmentary (Polley, 2005; Smith *et al.*, 2009; Schols *et al.*, 2020). Moreover, in the Anthropocene, habitat change, pollution, over-exploitation, climate change, and invasive species can all exacerbate the impact of diseases by directly altering parasite abundance and distribution (Lafferty and Kuris, 2005) or indirectly by reducing host population size and genetic diversity (Heard *et al.*, 2013; Budria and Candolin, 2014).

Combined with earlier work, we showed that human alteration to the environment has led to a surprising cascade of biological invasions, inducing parasite spillback and thereby threatening hippopotamus conservation efforts (**Figure 16**; Carolus *et al.*, 2019; Schols *et al.*, 2021; Schols and Huyse, 2024). A key aspect of this parasite spillback is the unusually high susceptibility of the intermediate snail host *P. columella* to *F. nyanzae*. Moreover, as this snail continues to invade new areas, animals and humans across Africa are at increased risk of fasciolosis (Grabner *et al.*, 2014;

Lounnas *et al.*, 2017; Schols *et al.*, 2021). One solution might be harvesting the invasive water hyacinth, which is the main substrate for *P. columella* in lake Kariba, for composting and agricultural fertilizer (Rohr *et al.*, 2023), controlling both invasive snails and their substrate. Especially since natural predators preferentially feed on native snails compared to *P. columella* (Appleton *et al.*, 2004). However, the vastness of the lake and broad distribution throughout Africa of both the plant and snail will undoubtedly complicate such an effort.

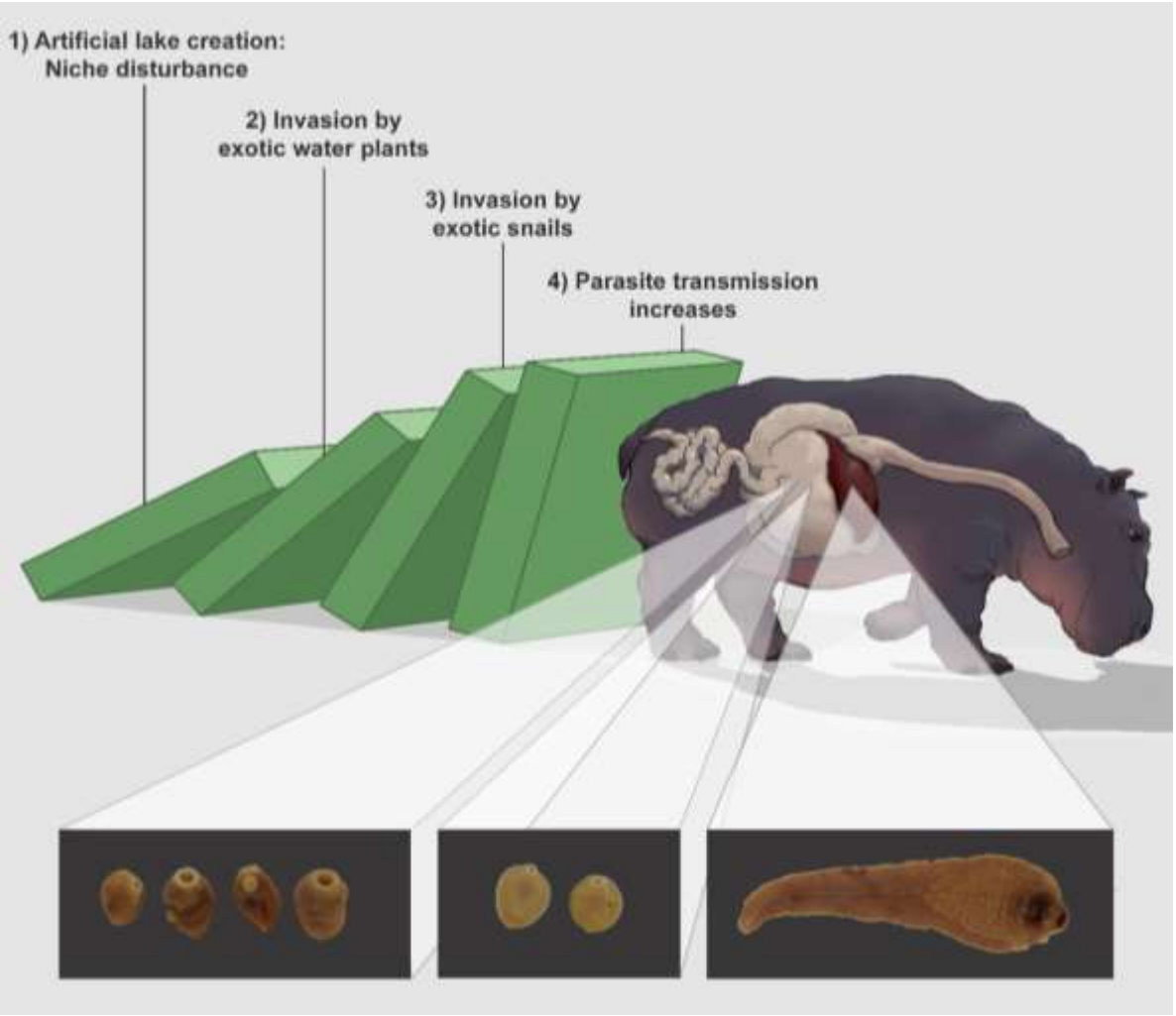

Figure 16: A cascade of biological invasions threatening *H. amphibius* conservation efforts.

The limited knowledge of the biology of *F. nyanzae* confounds impact estimates of the increased transmission through *P. columella* on populations of the hippopotamus (**Figure 17;** Schols *et al.*, 2021; Schols and Huyse, 2024). Infections by helminths can sometimes protect a host from subsequent infections (Karvonen *et al.*, 2004; Abruzzi and Fried, 2011; Rellstab *et al.*, 2013; Toledo and Fried, 2014). Indeed, up to a dozen liver flukes but not more can be retrieved from a single hippopotamus (reviewed in Schols and Huyse, 2024), suggesting some protective effect against subsequent infections. However, most of the time subsequent infections are worsened (Abruzzi and Fried, 2011; Louhi *et al.*, 2015). Although unknown for *F. nyanzae, F. gigantica* infection load can get problematic (Bindernagel, 1972 & own observations). As reports continue with unexplained mortalities of giraffes, buffaloes and eland antelopes across Zimbabwe, all occurring near artificial lakes where we confirmed the presence of *P. columella*, it raises suspicion of an unnoticed and ongoing epidemic threatening

wildlife conservation efforts across the country and beyond. Therefore, it will be imperative for future projects to focus on mapping the spread of invasive snails, their relative contribution to disease transmission, inventorying parasite biodiversity and quantifying the impact of these diseases on animal health for effective and sustainable wildlife conservation efforts (Kelly *et al.*, 2009; Thompson *et al.*, 2010; Mudavanhu *et al.*, 2024).

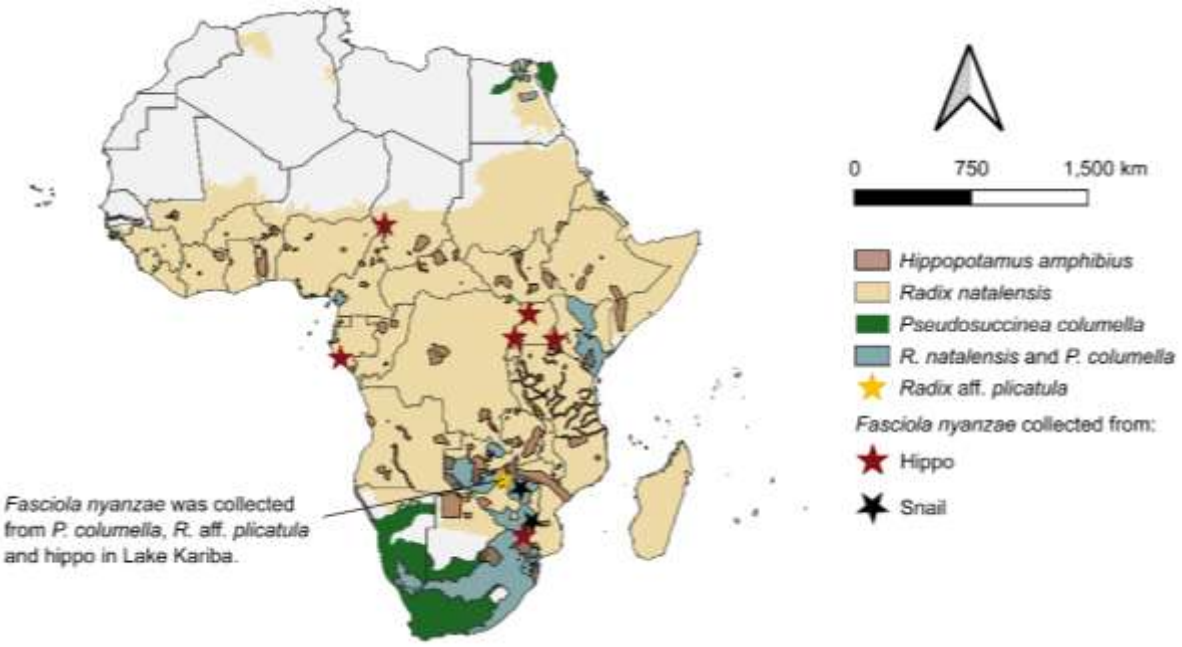

Figure 17: The historical and current records of the hippo-infecting liver fluke *Fasciola nyanzae* in relation to the geographical distribution of its definitive host *Hippopotamus amphibius,* and its intermediate freshwater snail hosts the native *Radix natalensis*, and the invasive exotic species *Pseudosuccinea columella*, and to a lesser extent *Radix* aff. *plicatula*.

An urgent need to increase monitoring efforts of trematode parasites and invasive snails, while also improving biological knowledge exists. It can be argued that this knowledge gap can only be filled through an integrative approach involving wildlife managers, hunters, citizen scientists, parasitologists, ecologists, and veterinarians. Increased financial investments and more experts would help considerably (Rodman and Cody, 2003; Vinarski, 2020). However, supplementary avenues can be explored to assess the impact of biological invasions on conservation efforts. First, citizen scientists can help expand the research area under study in addition to the sampling frequency (Ashepet *et al.*, 2021; Tumusiime *et al.*, 2024), which is vital to monitor the spread of invasive snails and to obtain temporal patterns. Second, the rise of approaches leveraging environmental DNA (eDNA) provides a rapid and efficient alternative to the standard and time-consuming sampling protocols to confirm the presence or absence of a species (Sengupta *et al.*, 2019; Douchet *et al.*, 2022). Third, the clinical assessment of veterinarians will be critical to quantify the impact of trematodiases on individual animals (Smith *et al.*, 2009). Fourth, opportunistic sampling, as in (Schols *et al.*, 2021), provides unique insights into the diseases affecting threatened wildlife species. However, it is not an easy feat and requires an intimate network with mutual trust between scientists, hunters and wildlife managers. Finally, close monitoring of wildlife populations will be vital to assess the effect at population level,

especially to detect more subtle patterns in populations that do not involve the (near) complete local extinction of a species. This is, however, not an easy task as it requires dedicated and knowledgeable personnel capable of monitoring the wildlife populations in such detail to allow this analysis.

Notably, parasites can also support healthy and diverse ecosystem functioning, driving genetic and phenotypic diversity (Gómez and Nichols, 2013; Stringer and Linklater, 2014; Wood and Vanhove, 2023). Consequently, the realization that parasite conservation holds value is gaining traction (Carlson *et al.*, 2020; Kwak *et al.*, 2020). Trematode parasites, like those of the common hippopotamus, stand out, as roughly 90% of the species still await discovery and formal description (Hechinger, 2023). The unknown stomach fluke, transmitted by the exotic *Radix* aff. *plicatula*, could be one of those undescribed species, as we were unsuccessful at identifying it despite an integrative taxonomic approach involving lifecycle information, morphology, and genetics (Will *et al.*, 2005; Schols *et al.*, 2020). Currently, the golden standard for stomach fluke identification relies on median sagittal sections and Scanning Electron Microscopy (SEM) imaging, both destructive approaches (Sey, 1991). Therefore, non-destructive identification methods will be imperative to leverage natural history collections and obtain historical perspectives. Micro-computed tomography (µCT), is a non-destructive 3D imaging technique that could offer this solution without altering the physical properties of a specimen (Faulwetter *et al.*, 2013). Moreover, because of the three-dimensional information, new diagnostic characteristics are frequently found (Ziegler *et al.*, 2010; Zimmermann *et al.*, 2011; Faulwetter *et al.*, 2013), which makes it especially valuable for stomach fluke taxonomy due to the complexity of species delimiting traits (Schols *et al.*, 2025a). Moreover, only a fraction of the described stomach fluke species have a genetic reference sequence available online, making the barcoding void especially pronounced for stomach flukes (Blasco-Costa *et al.*, 2016; Schols *et al.*, 2020). Therefore, parasite-parasite interactions within snails are undoubtedly going unnoticed since stomach flukes frequently interact with other trematode parasites in snail hosts, driving their transmission (Lim and Heyneman, 1972; Laidemitt *et al.*, 2019). The lack of knowledge of trematode biology, therefore, hinders biodiversity assessments, ecological studies, effective disease control and conservation efforts.

Future prospects and recommendations

For vector symbiont-manipulated control measures to work and be cost-efficient, replacement of wildtype vectors by resistant vectors should be feasible (Pinto *et al.*, 2021; Zimmermann *et al.*, 2024). Population replacement or persistence of the introduced vectors requires an augmented or at least comparable fitness relative to the native vectors (Manoj *et al.*, 2021; Pinto *et al.*, 2021). Resistance-competition trade-offs might make this difficult (Wolinska and King, 2009), yet has been shown to be surmountable for mosquitoes (Pinto *et al.*, 2021). Trematode infections impose a severe fitness cost on their snail host (King *et al.*, 2011; Faro *et al.*, 2013; Tavalire *et al.*, 2016; Paczesniak *et al.*, 2019), theoretically boosting the fitness of resistant snails. However, in the wild, other factors are at play since resistant snails rarely dominate local populations (Webster *et al.*, 2003; Alba *et al.*, 2019, 2022). Additionally, parasites and snails are in a constant evolutionary arms race (Jokela *et al.*, 2009). Combined with the strong selective pressure of releasing resistant snails, parasites might adapt and circumvent the introduced resistance factor. Therefore, taking an evolutionary perspective to the

snail-trematode interaction will be critical in combination with continuous work on discovering or developing additional resistance-inducing microbiota. Nevertheless, combined with integrated control measures involving improved sanitation and drug treatment, resistance-inducing microbiota might contribute to interrupting the transmission of detrimental trematode parasites.

The lack of putative resistance-inducing bacteria throughout the MicroResist project might have originated from methodological limitations that can be tackled in future projects. Although bacterial community analysis provides invaluable insights, it fails to unfold the mechanisms behind the host-microbiome interaction. The diversity analysis applied throughout this project sets the stage for investigating the bacterial functionality through a metagenomics approach. In combination, metabarcoding and metagenomics enable us to address pressing challenges in understanding the snail-microbiome-trematode interaction (Arribas *et al.*, 2016; Knight *et al.*, 2018). Sequencing whole bacterial genomes should provide these much-needed high-resolution functional insights (Knight *et al.*, 2018).

Analysing DNA extracts removes the 3D-world organisms live and interact in (Jahn *et al.*, 2016), which turns out to be critical given the limited diffusion distance of microbial metabolites (on average 15 µm) (van Tatenhove-Pel *et al.*, 2021). As a result, we undoubtedly investigated vast numbers of bacteria irrelevant to the role of resistance, which increased the noise and potentially confounded true signals in our dataset. Similar to Jahn *et al.* (2016) a tool capable of obtaining high-resolution *in situ* visualization of bacterial species will be groundbreaking. Not only will it enable us to study microbial ecology within the snail host, but it will also allow us to identify which bacterial species might be near the trematode parasite during key development stages. Specifically, it could be used to identify bacteria that are intimately linked to hemocytes during the first 24 hours of infection, a period critical for infection establishment (De Jong-Brink *et al.*, 2001). These hemocyte-associated bacteria could then be further investigated to identify putative hemocyte-modulating pathways. The high-resolution *in situ* visualization could also identify where exactly in the snail the observed microbial shifts, in response to trematode infection, occur as reported throughout this project. One such tool is currently in the pipeline but it depends on the full characterization of the host's bacterial community by sequencing the entire 16S and 23S rRNA genes (Martin Jahn pers. comm.), a technique that only recently became feasible (Johnson *et al.*, 2019). Nevertheless, it holds great promise and its inclusion in future experiments will undoubtedly contribute to a profound understanding of the snail-trematode-microbiome interaction.

Concluding remarks

The MicroResist project successfully explored the complex interplay between freshwater snails, their microbiomes, and trematode parasites, revealing that while microbiome transplantation alone did not confer resistance to infection in some *P. columella* populations, trematode infections consistently altered the snail-associated microbiome in species- and stage-specific ways. These microbial shifts may be linked to immune responses, tissue damage, or parasite-driven modulation, highlighting the need for further mechanistic studies. Although microbiome-mediated resistance remains elusive, the findings underscore the potential of targeting the microbiome—of either the host or the parasite—for future control strategies. Moving forward, integrating multi -omics perspectives, high-resolution

in situ visualization, and broader ecological and genetic background will be essential to uncover functional interactions and identify pathways of resistance for sustainable trematode control within a *One Health* framework.

Contribution to Policy-Relevant Scientific Support

The MicroResist project delivered a substantial scientific contribution by investigating the bacterial microbiome's influence on trematode resistance in freshwater snails—key intermediate hosts of parasitic diseases such as schistosomiasis and fasciolosis. These diseases have been increasing their foothold in Europe and are of growing global concern due to climate change, invasive species, and increased ecological disruption. The project developed and validated crucial experimental tools (e.g., germ-free snails, transplant protocols, sequencing and analysis pipelines), revealed species-specific microbiome dynamics under infection, identified ecological spillback risks from invasive snails, and provided the first record of the potentially neuropathogenic *Trichobilharzia regenti* in the Benelux an important finding related to the re-emergence of the disease across Europe and the planned opening of natural waterbodies for public swimming locations in Flanders (https://www.outdoorswimming.be/). Nevertheless, the project did not find direct evidence that microbiome composition alone confers resistance to trematodes in some populations of a single snail-parasite system. MicroResist provides Belgium with validated tools and scientific evidence:

- To guide and inform future research on microbiome-mediated disease interventions:
    - The developed transplant protocol will enable future research to define causal links of the microbiome with snail characteristics, such as, but not limited to, trematode resistance.
    - The sequencing and analysis pipelines form a strong bioinformatic framework for future studies.
    - The snail-associated microbiome is significantly affected by parasite exposure and infection status; consideration must be given to these factors in future experimental design and field monitoring efforts.
    - The identification of new targets for microbiome-mediated trematode control; key parasite development stages correspond to major shifts in the snail-associated microbiome.
- The importance of monitoring invasive species and parasite transmission, as evidenced by:
    - The impact on conservation strategies of threatened species such as the common hippopotamus, *Hippopotamus amphibius*.
    - The impact on human disease epidemiology with a new and potentially neuropathogenic swimmer's itch agent for the Benelux.

<u>Concrete Recommendations</u>

1. Prioritize funding for One Health-compatible snail control strategies: Belgian and international development funding should support further studies using microbiome-focused, nature-based control strategies which aim to identify microbiota configurations that enhance resistance in native snail

populations. For example, microbiome transplant protocols, as we describe here, can be used to experimentally test and obtain resistant snail lines for ecological interventions as done for mosquito-borne diseases (Wang *et al.*, 2012; Gomes *et al.*, 2017; Pinto *et al.*, 2021; Shi *et al.*, 2023). These can inform ecological engineering efforts such as habitat restoration that favors resistant host microbiomes. For example, our tool recently revealed the microbiome-mediated adaptation of the native *Cipangopaludina chinensis* to *Pomacea canaliculata* invasion, indicating that microbiome transplants could be a valuable conservation method for threatened native freshwater snails (Liu *et al.*, 2025). This could also pave the way for developing resistance-inducing inoculants suitable for field application. We suggest a functional rather than compositional approach, while considering the actual bio-engineering of resistance-inducing microbes aligned with EU legislation on genetically modified organisms and environmental safety.

2. Ensure the preservation of museum collections and biobanks for retrospective disease ecology studies: The MicroResist project valorized preserved museum samples over time and space on multiple occasions. Given the precarious situation of funding for natural history collections, Belgium should reconsider its funding strategy to enable similar impactful research in the future. This approach can improve understanding of long-term trends in host-parasite-microbiome interactions and biodiversity as a whole. Strategic funding should be allocated to collection management, collection expansion, digitization, and metadata enrichment.

3. Revise public health risk assessments to include parasite spillback to wildlife from invasive hosts: Belgian and EU conservation policies should broaden their disease risk frameworks to include cross-species infection pathways in biodiversity and conservation status assessments, especially in protected or rewilding areas. Risk models should include invasive host ecology and parasitological surveillance as they can drastically affect population health and thereby conservation efforts, as demonstrated by the Lake Kariba case study. Moreover, disease monitoring is essential for guiding policy and enabling early intervention for outbreaks of veterinary [e.g. fasciolosis (Pointier *et al.*, 2007; Lounnas *et al.*, 2017)] and human health importance [e.g. schistosomiasis (Mulero *et al.*, 2019), swimmer's itch (Soldánová *et al.*, 2016; Schols *et al.*, 2024)] under the Animal Health Law framework. This includes monitoring the invasive and highly compatible snail *Pseudosuccinea columella* in southern France, which may exacerbate *Fasciola hepatica* transmission and burden ruminant farming; tracking the now-endemic urinary schistosomiasis in Corsica to anticipate spread to mainland Europe; and mapping swimmer's itch agents across Belgium, especially in light of the (re-)emergence of cases and the recent detection of the potentially neuropathogenic *T. regenti*. Enhanced biological understanding and targeted surveillance will support control efforts and inform the European Centre for Disease Prevention and Control (ECDC).

4. Strengthen international collaboration for parasite monitoring: Belgium should support collaborative capacity building in disease vector monitoring, molecular parasite detection, and microbiome sequencing. This would enhance global disease surveillance within the One Health framework, benefitting Belgium's preparedness to disease emergence. The MicroResist project set up a core network to which additional partners can be recruited and from which surveillance programs can be established. Moreover, Belgium should also consider monitoring snail-borne diseases at the national level to obtain baseline information on potential disease vectors, the presence of invasive species potentially shaping disease transmission, and provide an early warning system for upcoming disease outbreaks and thereby also contributing to the international surveillance network. As an

added benefit, it will directly contribute to the environmental monitoring programs under the EU Water Framework Directive by mapping aquatic biodiversity.

Side results

The MicroResist project enabled the complete PhD project of Dr. Ruben Schols, and the support of chapters of two PhD projects (PhD candidate Emilie Gossens, Dr. Aspire Mudavanhu), five MSc students (MSc Biochemistry Ruben Put, MSc Biology Arthur Piot, MSc Biology Emilie Goossens, MSc Sustainable Development Shandré van der Merwe, and MSc Biology Kalle Lambaerts), three internships (MSc Biology Kalle Lambaerts, MSc Sustainable Development Larissa Bonifacio, and MSc Biology Ellen Vandenbussche), and one student worker (MSc Biology Emilie Goossens). Additionally, it enabled us to train the next generation of students in our lab (https://x.com/rubenschols/status/1769666759173210421?s=20; PhD candidate Cecilia Wangari Wambui, MSc sustainable Development Hannah Njiriku Mwangi, MSc Biology Mila Viaene, and MSc Benjamin André). Furthermore, two scientific visitors from Uganda (Dr. Julius Tumusiime) and Congo (PhD candidate MD Germain Kapour) were supported during their molecular work in the laboratory. As didactic material the MicroResist generated an animation video (see next section) and produced a novel technique to digitize and 3D print natural history collection specimens using µCT scanning. The resulting 3D models are highly appreciated by the general public and students during seminars and outreach activities on trematode parasites. We also connected with scientists from across the world and established (inter)national collaborative research lines with RMCA involving several institutes and universities in Belgium (ITM, UHasselt, UGhent, KU Leuven, and RBINS) and abroad (France: University of Perpignan, University of Montpellier; South Africa: University of Pretoria, University of Limpopo; Zimbabwe: Lake Kariba Research Station, University of Zimbabwe, Bindura University), strengthening the (inter)national presence and recognition of Belgian federal institutes.

Additionally, the MicroResist project enabled field monitoring of a swimmers itch outbreak in Kampenhout, leading to the detection of a new and potentially neuropathogenic species for the Benelux (*Trichobilharzia regenti;* Schols *et al.*, 2024). This finding contributes to the baseline knowledge on swimmers itch agents in Belgium and may facilitate future monitoring efforts at the national level. This becomes increasingly important given the health and economic implications combined with the (re-)emergence of swimmers itch across Europe (Mas-Coma *et al.*, 2009b; Soldánová *et al.*, 2013; Christiansen *et al.*, 2016).

5. DISSEMINATION AND VALORISATION

Public outreach activities

Considerable scientific outreach efforts have been undertaken by conceptualizing and overseeing the creation of an animation video on the MicroResist project permanently available on YouTube (over 340 views). This video is currently still used to inspire students at KU Leuven and visitors at RMCA. It has also been shown at the Empowering Biodiversity Research (EBR) II conference - Tervuren, Belgium, 24–25 May 2022 (an estimated 200 participants). We supervised the event “Meet the scientist: Zieke slakken (onder)zoeken @RMCA 2023” by collecting and demonstrating live snails and parasites to the general public. We also gave two guest lecture to 3rd-year students of the "Parasitology and One

Health" course at UHasselt while visiting our federal institute. We also wrote two popularizing articles in EOS and featured in articles in *EOS, de Gazet van Antwerpen, National Geographic, Het Laatste Nieuws,* and *Het Belang van Limburg*. Finally, the public defence of the MicroResist project by Ruben Schols attracted over 90 attendees across all age groups and backgrounds.

Presentations and posters at international conferences

Schols, R., Hammoud, C., Maes, T., Senghor, B., Vanoverberghe, I., Huyse, T., Decaestecker, E. (2024). The snail-trematode-microbiome tripartite interaction: From lab manipulations to the field. Presented at SymbNET: Host-Microbe Symbiosis, Olhão, Portugal.

Schols, R. (2024). How the enigmatic hippo may be threatened by the globally invasive snail *Pseudosuccinea columella* - Could microbiome transplants provide the solution? Presented at the Seminar, Perpignan, France.

Schols, R., Hammoud, C., Maes, T., Senghor, B., Vanoverberghe, I., Huyse, T., Decaestecker, E. (2023). The snail-trematode-microbiome tripartite interaction: From lab manipulations to the field. Presented at the Belgian Society for Parasitology and Protistology, Leuven, Belgium.

Schols, R., Hammoud, C., Maes, T., Senghor, B., Isabel, V., Huyse, T., Decaestecker, E. (2023). The snail-trematode-microbiome tripartite interaction: From lab manipulations to the field. Presented at the American Society of Tropical Medicine and Hygiene, Chicago, USA.

Schols, R., Smitz, N., Vanderheyden, A., Huyse, T. (2023). Swimmer's itch in Belgium: A first record of *Tricholbilharzia regenti*. Presented at The Danish Society of Parasitology, Copenhagen, Denmark.

Schols, R., Decaestecker, E., Huyse, T. (2022). MicroResist - The influence of snail host microbiome in trematode parasite resistance. Presented at the Closing symposium 'Ecology and Evolution of the microbiome', Kortrijk, Belgium.

Schols, R., Decaestecker, E., Huyse, T. (2022). MICRORESIST - The influence of snail host microbiome in trematode parasite resistance. Presented at the Belgian Society for Parasitology & Protistology, Leuven, Belgium.

Schols, R., Hammoud, C., Put, R., Piot, A., Maes, T., Senghor, B., Decaestecker, E., Huyse, T. (2022). Towards a better understanding of the snail-trematode-microbiome interactions driving schistosome transmission in Senegal. Presented at the Benelux Congress Of Zoology 2022, Kortrijk, Belgium.

Schols, R., Put, R., Piot, A., Hammoud, C., Maes, T., Senghor, B., Decaestecker, E., Huyse, T. (2022). Towards a better understanding of the snail-trematode-microbiome interactions driving schistosome transmission in Senegal. Presented at The 15th International Congress of Parasitology, Copenhagen, Denmark.

Schols, R., Huyse, T., Decaestecker, E. (2021). The influence of snail host microbiome in trematode parasite resistance. Presented at The 15th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases, Online.

Schols, R., Huyse, T., Decaestecker, E. (2021). MicroResist - The influence of snail host microbiome in trematode parasite resistance. Presented at the Ecology and Evolution of Parasites and Infections, 5th summer workshop, Antwerp, Belgium.

Schols, R., Carolus, H., Hammoud, C., Muzarabani, K., Barson, M., Huyse, T. (2021). Invasive snails, parasite spillback and potential parasite spillover drive parasitic diseases of *Hippopotamus amphibius* in artificial lakes of Zimbabwe. Presented at the 49th Annual Virtual Conference of the Parasitological Society of Southern Africa, online.

6. PUBLICATIONS

Peer review

1. Schols, R., Hammoud, C., Bisschop, K., Vanoverberghe, I., Huyse, T. & Decaestecker, E. Schistosome species, parasite development, and co-infection combinations determine microbiome dynamics in the snail *Biomphalaria glabrata*, 2025, PREPRINT. Available at Research Square [https://doi.org/10.21203/rs.3.rs-6646903/v1] under consideration at *Animal Microbiome*
2. Schols, R., Henrard, A., Brecko, J., Mudavanhu, A., Goossens, E., Steffanie, N., Clegg, S., Vanhove, M.P.M. & Huyse, T. Innovating stomach fluke identification: an integrative approach combining Micro-CT imaging and molecular tools. *International Journal for Parasitology*. (2025) in press.
3. Maes, T., Verheyen, J., Senghor, B., Mudavanhu, A., Schols, R., Hellemans, B., Geslain, E., Volckaert, F. A. M., Gante, H. F. & Huyse, T. First evidence of a genetic basis for thermal adaptation in a schistosome host snail. *Ecol. Monogr.* (2025) **95**, e70006.
4. Kapour, G. K. K., Wangari, C. W., Schols, R., Madinga, J. N., Mitashi, P. M. & Huyse, T. First record of an alien species of *Amerianna* Strand, 1928 (Gatropoda: Hygrophila: Planorbidae) in the Kimpese region, Democratic Republic of Congo. *Folia Malacol.* (2024) **32**, 129–135.
5. Schols, R. & Huyse, T. *Fasciola nyanzae*. *Trends Parasitol.* (2024) **40**, 527–528.
6. Schols, R., Smitz, N., Vanderheyden, A. & Huyse, T. Expanding the swimmer's itch pool of the Benelux: a first record of the neurotropic *Trichobilharzia regenti* and potential link to human infection. *Parasites and Vectors* (2024) **17**, 1–11.
7. Mudavanhu, A., Schols, R., Goossens, E., Nhiwatiwa, T., Manyangadze, T., Brendonck, L. & Huyse, T. One Health monitoring reveals invasive freshwater snail species, new records, and undescribed parasite diversity in Zimbabwe. *Parasites and Vectors* (2024) **17**, 1–17.
8. Mudavanhu, A., Goossens, E., Schols, R., Manyangadze, T., Nhiwatiwa, T., Lemmens, P., Huyse, T. & Brendonck, L. Ecosystem links: Anthropogenic activities, environmental variables, and macrophytes structure snail preferences in man-made waterbodies. *Sci. Total Environ.* (2024) **954**, 176394.
9. Schols, R., Vanoverberghe, I., Huyse, T. & Decaestecker, E. Host-bacteriome transplants of the schistosome snail host *Biomphalaria glabrata* reflect species-specific associations. *FEMS Microbiol. Ecol.* (2023) **99**, fiad101.

10. Muzarabani, K. C., Carolus, H., Schols, R., Hammoud, C., Barson, M. & Huyse, T. An update on snail and trematode communities in the Sanyati Basin of Lake Kariba: New snail and trematode species but no human schistosomes. *Parasitol. Int.* (2023) 102830.
11. Tumusiime, J., Kagoro-Rugunda, G., Tolo, C. U., Namirembe, D., Schols, R., Hammoud, C., Albrecht, C. & Huyse, T. An accident waiting to happen? Exposing the potential of urogenital schistosomiasis transmission in the Lake Albert region, Uganda. *Parasit. Vectors* (2023) **16**, 398.
12. Stock, W., Callens, M., Houwenhuyse, S., Schols, R., Goel, N., Coone, M., Theys, C., Delnat, V., Boudry, A. & Eckert, E. M. Human impact on symbioses between aquatic organisms and microbes. *Aquat. Microb. Ecol.* (2021) **87**, 113–138.
13. Schols, R., Carolus, H., Hammoud, C., Muzarabani, K. C., Barson, M. & Huyse, T. Invasive snails, parasite spillback, and potential parasite spillover drive parasitic diseases of *Hippopotamus amphibius* in artificial lakes of Zimbabwe. *BMC Biol.* (2021) **19**, 160.
14. Schols, R., Mudavanhu, A., Carolus, H., Hammoud, C., Muzarabani, K. C., Barson, M. & Huyse, T. Exposing the Barcoding Void: An Integrative Approach to Study Snail-Borne Parasites in a One Health Context. *Frontiers in Veterinary Science* at (2020) **7**, 1046.

All listed papers except references 2, 5, 8, and 10 are Open Access publications freely accessible online. The four remaining subscription based publications are added in Annex and remain freely accessible upon request from the authors.

Other

Schols, R., Lotte, C. (contr.) (2022). Infographic: How bacteria can help us fight DEADLY parasitic diseases! MicroResist. (URL)

Reitsma, D., Schols, R. (contr.), Huyse, T. (contr.) (2021). Ongenode gasten. *National Geographic. Nederland-België*, 16-16.

Mudavanhu, A., Schols, R., Put, R. (2021). On freshwater snails, their microbiome and parasite diversity: The epidemiological implications of invasive snails for Zimbabwe. (URL)

Huyse, T., Carolus, H., Schols, R. (2020). Stuwdammen zijn broeihaarden voor infectieziektes. *Eos Magazine*, 70-74. (URL)

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ANNEXES

References

Abebe, F., Erko, B., Gemetchu, T. and Gundersen, S.G. (2005) 'Control of *Biomphalaria pfeifferi* population and schistosomiasis transmission in Ethiopia using the soap berry endod (*Phytolacca dodecandra*), with special emphasis on application methods', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 99(10), pp. 787–794. Available at: https://doi.org/10.1016/j.trstmh.2005.04.013.

Abruzzi, A. and Fried, B. (2011) 'Chapter 1 - Coinfection of Schistosoma (Trematoda) with Bacteria, Protozoa and Helminths', in D. Rollinson and S.I. Hay (eds) *Advances in Parasitolog*. Academic Press, pp. 1–85. Available at: https://doi.org/10.1016/B978-0-12-391429-3.00005-8.

Agatsuma, T., Arakawa, Y., Iwagami, M., Honzako, Y., Cahyaningsih, U., Kang, S.-Y. and Hong, S.-J. (2000) 'Molecular evidence of natural hybridization between *Fasciola hepatica* and *F. gigantica*', *Parasitology International*, 49(3), pp. 231–238. Available at: https://doi.org/https://doi.org/10.1016/S1383-5769(00)00051-9.

Alba, A., Vázquez, A.A., Hernández, H., Sánchez, J., Marcet, R., Figueredo, M., Sarracent, J. and Fraga, J. (2015a) 'A multiplex PCR for the detection of *Fasciola hepatica* in the intermediate snail host *Galba cubensis*.', *Veterinary parasitology*, 211(3–4), pp. 195–200. Available at: https://doi.org/10.1016/j.vetpar.2015.05.012.

Alba, A., Hernández, H.M., Marcet, R., Vázquez, A.A., Figueredo, M., Sánchez, J., Otero, O. and Sarracent, J. (2015b) 'A novel monoclonal antibody-based immunoenzymatic assay for epidemiological surveillance of the vector snails of Fasciola hepatica (Trematoda: Digenea)', *International Journal for Parasitology*, 45(2), pp. 113–119. Available at: https://doi.org/10.1016/j.ijpara.2014.09.007.

Alba, A., Vázquez, A.A., Sánchez, J., Duval, D., Hernández, H.M., Sabourin, E., Vittecoq, M., Hurtrez-Boussés, S. and Gourbal, B. (2018) '*Fasciola hepatica-Pseudosuccinea columella* interaction: Effect of increasing parasite doses, successive exposures and geographical origin on the infection outcome of susceptible and naturally-resistant snails from Cuba', *Parasites and Vectors*, 11(1), pp. 1–9. Available at: https://doi.org/10.1186/s13071-018-3155-3.

Alba, A., Vázquez, A.A., Sánchez, J., Lounnas, M., Pointier, J.-P., Hurtrez-Boussès, S. and Gourbal, B. (2019) 'Patterns of distribution, population genetics and ecological requirements of field-occurring resistant and susceptible Pseudosuccinea columella snails to Fasciola hepatica in Cuba', *Scientific Reports*, 9(1), p. 14359. Available at: https://doi.org/10.1038/s41598-019-50894-7.

Alba, A., Duval, D., Sánchez, J., Pérez, A.B., Pinaud, S., Galinier, R., Vázquez, A.A. and Gourbal, B. (2020) 'The immunobiological interplay between *Pseudosuccinea columella* resistant/susceptible snails with *Fasciola hepatica*: Hemocytes in the spotlight', *Developmental and Comparative Immunology*, 102(January 2019), p. 103485. Available at: https://doi.org/10.1016/j.dci.2019.103485.

Alba, A., Vázquez, A.A., Sánchez, J. and Gourbal, B. (2022) 'Immunological Resistance of *Pseudosuccinea columella* Snails From Cuba to *Fasciola hepatica* (Trematoda) Infection: What We Know and Where We Go on Comparative Molecular and Mechanistic Immunobiology, Ecology and Evolution', *Frontiers in Immunology*, p. 794186.

Alba, A., Vazquez, A.A. and Hurtrez-Boussès, S. (2021) 'Towards the comprehension of fasciolosis (re-)emergence: an integrative overview.', *Parasitology*, 148(4), pp. 385–407. Available at: https://doi.org/10.1017/S0031182020002255.

Alberdi, A., Aizpurua, O., Bohmann, K., Zepeda-Mendoza, M.L. and Gilbert, M.T.P. (2016) 'Do

Vertebrate Gut Metagenomes Confer Rapid Ecological Adaptation?', *Trends in Ecology & Evolution*, 31(9), pp. 689–699. Available at: https://doi.org/https://doi.org/10.1016/j.tree.2016.06.008.

Allan, E.R.O., Tennessen, J.A., Sharpton, T.J. and Blouin, M.S. (2018) 'Allelic Variation in a Single Genomic Region Alters the Microbiome of the Snail <i>Biomphalaria glabrata<i>', *Journal of Heredity*, 109(5), pp. 604–609. Available at: https://doi.org/10.1093/jhered/esy014.

Allan, F., Rollinson, D., Smith, J.E. and Dunn, A.M. (2009) 'Host choice and penetration by *Schistosoma haematobium* miracidia', *Journal of Helminthology*, 83(1), pp. 33–38. Available at: https://doi.org/10.1017/S0022149X08073628.

Allen, D.J., Brooks, E.G.E., Darwall, W.R.T., Harrison, I.J., Holland, R.A. and Smith, K.G. (2011) *The diversity of life in African freshwaters : under water, under threat : an analysis of the status and distribution of freshwater species throughout mainland Africa*. Available at: https://www.iucnredlist.org/resources/darwall-etal-2011.

Anders, K.L. *et al.* (2018) 'The AWED trial (Applying *Wolbachia* to Eliminate Dengue) to assess the efficacy of *Wolbachia*-infected mosquito deployments to reduce dengue incidence in Yogyakarta, Indonesia: study protocol for a cluster randomised controlled trial', *Trials*, 19(1), p. 302. Available at: https://doi.org/10.1186/s13063-018-2670-z.

Anyolitho, M.K., Poels, K., Huyse, T., Tumusiime, J., Mugabi, F., Tolo, C.U., Masquillier, C. and Nyakato, V.N. (2022) 'Knowledge, attitudes, and practices regarding schistosomiasis infection and prevention: A mixed-methods study among endemic communities of western Uganda', *PLoS Neglected Tropical Diseases*, 16(2), pp. 1–21. Available at: https://doi.org/10.1371/journal.pntd.0010190.

Anyolitho, M.K., Huyse, T., Masquillier, C., Nyakato, V.N. and Poels, K. (2024) 'Empowering communities through citizen science and participatory action research: implementation of a schistosomiasis communication campaign in Uganda', *Humanities and Social Sciences Communications*, 11(1), p. 227. Available at: https://doi.org/10.1057/s41599-024-02714-1.

Appleton, C.C., Forbes, A.T. and Demetriades, N.T. (2009) 'The occurrence, bionomics and potential impacts of the invasive freshwater snail *Tarebia granifera* (Lamarck, 1822) (Gastropoda: Thiaridae) in South Africa', *Zoologische Mededelingen*, 83(4), pp. 525–536.

Appleton, C.C., Hofkin, B. V. and Baijnath, A. (2004) 'Macro-invertebrate predators of freshwater pulmonate snails in Africa, with particular reference to Appasus grassei (Heteroptera) and Procambarus clarkii (Decapoda)', *African Journal of Aquatic Science*, 29(2), pp. 185–193. Available at: https://doi.org/10.2989/16085910409503809.

Archie, E.A. and Theis, K.R. (2011) 'Animal behaviour meets microbial ecology', *Animal Behaviour*, 82(3), pp. 425–436. Available at: https://doi.org/10.1016/j.anbehav.2011.05.029.

Arribas, P., Andújar, C., Hopkins, K., Shepherd, M. and Vogler, A.P. (2016) 'Metabarcoding and mitochondrial metagenomics of endogean arthropods to unveil the mesofauna of the soil', *Methods in Ecology and Evolution*, 7(9), pp. 1071–1081. Available at: https://doi.org/https://doi.org/10.1111/2041-210X.12557.

Ashepet, M.G., Jacobs, L., Van Oudheusden, M. and Huyse, T. (2021) 'Wicked Solution for Wicked Problems: Citizen Science for Vector-Borne Disease Control in Africa', *Trends in Parasitology*, 37(2), pp. 93–96. Available at: https://doi.org/10.1016/j.pt.2020.10.004.

Babbitt, C.R., Laidemitt, M.R., Mutuku, M.W., Oraro, P.O., Brant, S. V., Mkoji, G.M. and Loker, E.S. (2023) '*Bulinus* snails in the Lake Victoria Basin in Kenya: Systematics and their role as hosts for

schistosomes', *PLoS Neglected Tropical Diseases*, 17(2), pp. 1–25. Available at: https://doi.org/10.1371/journal.pntd.0010752.

Bai, L., Wang, L., Vega-Rodríguez, J., Wang, G. and Wang, S. (2019) 'A gut symbiotic bacterium serratia marcescens renders mosquito resistance to plasmodium infection through activation of mosquito immune responses', *Frontiers in Microbiology*, 10, p. 1580. Available at: https://doi.org/10.3389/fmicb.2019.01580.

Baldassarre, L., Reitzel, A.M. and Fraune, S. (2023) 'Genotype–environment interactions determine microbiota plasticity in the sea anemone *Nematostella vectensis*', *PLOS Biology*, 21(1), p. e3001726. Available at: https://doi.org/10.1371/journal.pbio.3001726.

Bankers, L., Dahan, D., Neiman, M., Adrian-Tucci, C., Frost, C., Hurst, G.D.D. and King, K.C. (2021) 'Invasive freshwater snails form novel microbial relationships', *Evolutionary Applications*, 14(3), pp. 770–780. Available at: https://doi.org/https://doi.org/10.1111/eva.13158.

Baquero, F. and Nombela, C. (2012) 'The microbiome as a human organ.', *Clinical microbiology and infection*. England, pp. 2–4. Available at: https://doi.org/10.1111/j.1469-0691.2012.03916.x.

Berg, G. *et al.* (2020) 'Microbiome definition re-visited: old concepts and new challenges', *Microbiome*, 8(1), p. 103. Available at: https://doi.org/10.1186/s40168-020-00875-0.

Bindernagel, J.A. (1972) 'Liver Fluke *Fasciola gigantica* In African Buffalo And Antelopes In Uganda, East Africa', *Journal of Wildlife Diseases*, 8(4), pp. 315–317. Available at: https://doi.org/10.7589/0090-3558-8.4.315.

Bisschop, K., Kortenbosch, H.H., van Eldijk, T.J.B., Mallon, C.A., Salles, J.F., Bonte, D. and Etienne, R.S. (2022) 'Microbiome Heritability and Its Role in Adaptation of Hosts to Novel Resources', *Frontiers in Microbiology*, 13.

Blasco-Costa, I., Cutmore, S.C., Miller, T.L. and Nolan, M.J. (2016) 'Molecular approaches to trematode systematics: "best practice" and implications for future study', *Systematic Parasitology*, 93(3), pp. 295–306. Available at: https://doi.org/10.1007/s11230-016-9631-2.

Van Bocxlaer, B., Clewing, C., Mongindo Etimosundja, J.-P., Kankonda, A., Wembo Ndeo, O. and Albrecht, C. (2015) 'Recurrent camouflaged invasions and dispersal of an Asian freshwater gastropod in tropical Africa', *BMC Evolutionary Biology*, 15(1), p. 33. Available at: https://doi.org/10.1186/s12862-015-0296-2.

Böhm, M. *et al.* (2021) 'The conservation status of the world's freshwater molluscs', *Hydrobiologia*, 848(12), pp. 3231–3254. Available at: https://doi.org/10.1007/s10750-020-04385-w.

Boissier, J. *et al.* (2016) 'Outbreak of urogenital schistosomiasis in Corsica (France): an epidemiological case study', *The Lancet Infectious Diseases*, 16(8), pp. 971–979. Available at: https://doi.org/10.1016/S1473-3099(16)00175-4.

Bosco, N. and Noti, M. (2021) 'The aging gut microbiome and its impact on host immunity', *Genes and Immunity*, 22(5–6), pp. 289–303. Available at: https://doi.org/10.1038/s41435-021-00126-8.

Bourne, M.E. *et al.* (2023) *Parasitism causes changes in caterpillar odours and associated bacterial communities with consequences for host-location by a hyperparasitoid*, *PLoS Pathogens*. Available at: https://doi.org/10.1371/journal.ppat.1011262.

Braks, M. *et al.* (2014) 'Vector-Borne Disease Intelligence: Strategies to Deal with Disease Burden and Threats', *Frontiers in Public Health*, 2. Available at: https://doi.org/10.3389/fpubh.2014.00280.

Brant, S. V, Morgan, J.A.T., Mkoji, G.M., Snyder, S.D., Rajapakse, R.P.V.J. and Loker, E.S. (2006) 'An approach to revealing blood fluke life cycles, taxonomy, and diversity: Provision of key reference data including DNA sequence from single life cycle stages', *Journal of Parasitology*, 92(1), pp. 77–88. Available at: https://doi.org/10.1645/GE-3515.1.

Brestoff, J.R. and Artis, D. (2013) 'Commensal bacteria at the interface of host metabolism and the immune system', *Nature Immunology*, 14(7), pp. 676–684. Available at: https://doi.org/10.1038/ni.2640.

Brinker, P., Fontaine, M.C., Beukeboom, L.W. and Falcao Salles, J. (2019) 'Host, Symbionts, and the Microbiome: The Missing Tripartite Interaction', *Trends in Microbiology*, 27(6), pp. 480–488. Available at: https://doi.org/10.1016/j.tim.2019.02.002.

Brönmark, C. (1989) 'Interactions Between Epiphytes, Macrophytes And Freshwater Snails: A Review', *Journal of Molluscan Studies*, 55(2), pp. 299–311. Available at: https://doi.org/10.1093/mollus/55.2.299.

Budria, A. and Candolin, U. (2014) 'How does human-induced environmental change influence host-parasite interactions?', *Parasitology*, 141(4), pp. 462–474. Available at: https://doi.org/10.1017/S0031182013001881.

Callens, M., Watanabe, H., Kato, Y., Miura, J. and Decaestecker, E. (2018) 'Microbiota inoculum composition affects holobiont assembly and host growth in *Daphnia*', *Microbiome*, 6(1), p. 56. Available at: https://doi.org/10.1186/s40168-018-0444-1.

Carlson, C.J. *et al.* (2020) 'A global parasite conservation plan', *Biological Conservation*, 250, p. 108596. Available at: https://doi.org/https://doi.org/10.1016/j.biocon.2020.108596.

Carolus, H., Muzarabani, K.C., Hammoud, C., Schols, R., Volckaert, F.A.M., Barson, M. and Huyse, T. (2019) 'A cascade of biological invasions and parasite spillback in man-made Lake Kariba', *Science of the Total Environment*, 659, pp. 1283–1292. Available at: https://doi.org/10.1016/j.scitotenv.2018.12.307.

Čaušević, S., Dubey, M., Morales, M., Salazar, G., Sentchilo, V., Carraro, N., Ruscheweyh, H.J., Sunagawa, S. and van der Meer, J.R. (2024) 'Niche availability and competitive loss by facilitation control proliferation of bacterial strains intended for soil microbiome interventions', *Nature Communications*, 15(1), pp. 1–19. Available at: https://doi.org/10.1038/s41467-024-46933-1.

Chandiwana, S.K., Bradley, M. and Woolhouse, M.E.J. (1991) 'Factors affecting the intensity of reinfection with *Schistosoma haematobium* following treatment with praziquantel', *Parasitology*, 102(1), pp. 73–83. Available at: https://doi.org/10.1017/S0031182000060364.

Charlier, J., van der Voort, M., Kenyon, F., Skuce, P. and Vercruysse, J. (2014) 'Chasing helminths and their economic impact on farmed ruminants.', *Trends in parasitology*, 30(7), pp. 361–367. Available at: https://doi.org/10.1016/j.pt.2014.04.009.

Chen, L., Li, S., Xiao, Q., Lin, Y., Li, X., Qu, Y., Wu, G. and Li, H. (2021) 'Composition and diversity of gut microbiota in *Pomacea canaliculata* in sexes and between developmental stages', *BMC Microbiology*, 21(1), p. 200. Available at: https://doi.org/10.1186/s12866-021-02259-2.

Chernin, E. (1957) 'A Method of Securing Bacteriologically Sterile Snails (*Australorbis glabratus*).', *Proceedings of the Society for Experimental Biology and Medicine*, 96(1), pp. 204–210. Available at: https://doi.org/10.3181/00379727-96-23433.

Chernin, E. (1960) 'Infection of Australorbis glabratus with Schistosoma mansoni under

Bacteriologically Sterile Conditions.', *Proceedings of the Society for Experimental Biology and Medicine*, 105(2), pp. 292–296. Available at: https://doi.org/10.3181/00379727-105-26088.

Chernin, E. and Schork, A.R. (1959) 'Growth in axenic culture of the snail, Australorbis glabratus.', *American journal of hygiene*, 69(2), pp. 146–160. Available at: https://doi.org/10.1093/oxfordjournals.aje.a119988.

Chernov, A. V, Reyes, L., Xu, Z., Gonzalez, B., Golovko, G., Peterson, S., Perucho, M., Fofanov, Y. and Strongin, A.Y. (2015) 'Mycoplasma CG- and GATC-specific DNA methyltransferases selectively and efficiently methylate the host genome and alter the epigenetic landscape in human cells.', *Epigenetics*, 10(4), pp. 303–318. Available at: https://doi.org/10.1080/15592294.2015.1020000.

Chevalier, F.D. *et al.* (2019) 'Oxamniquine resistance alleles are widespread in Old World *Schistosoma mansoni* and predate drug deployment', *PLoS Pathogens*, 15(10), pp. 1–25. Available at: https://doi.org/10.1371/journal.ppat.1007881.

Chevalier, F.D., Diaz, R., McDew-White, M., Anderson, T.J.C. and Le Clec'h, W. (2020) 'The hemolymph of *Biomphalaria* snail vectors of schistosomiasis supports a diverse microbiome', *Environmental Microbiology*, 22(12), pp. 5450–5466. Available at: https://doi.org/https://doi.org/10.1111/1462-2920.15303.

Chiarello, M., Bucholz, J.R., McCauley, M., Vaughn, S.N., Hopper, G.W., Sánchez González, I., Atkinson, C.L., Lozier, J.D. and Jackson, C.R. (2022) 'Environment and Co-occurring Native Mussel Species, but Not Host Genetics, Impact the Microbiome of a Freshwater Invasive Species (*Corbicula fluminea*).', *Frontiers in microbiology*, 13, p. 800061. Available at: https://doi.org/10.3389/fmicb.2022.800061.

Christiansen, A.Ø., Olsen, A., Buchmann, K., Kania, P.W., Nejsum, P. and Vennervald, B.J. (2016) 'Molecular diversity of avian schistosomes in Danish freshwater snails.', *Parasitology research*, 115(3), pp. 1027–1037. Available at: https://doi.org/10.1007/s00436-015-4830-3.

Chrostek, E., Marialva, M.S.P., Yamada, R., O'Neill, S.L. and Teixeira, L. (2014) 'High anti-viral protection without immune upregulation after interspecies *Wolbachia* transfer', *PLoS ONE*, 9(6), pp. 1–7. Available at: https://doi.org/10.1371/journal.pone.0099025.

Chrostek, E., Martins, N. and Marialva, M.S. (2021) 'Wolbachia-Conferred Antiviral Protection Is Determined by Developmental Temperature', *mBio*, 12(5). Available at: https://doi.org/10.1128/mbio.02923-20.

Ciceri, D. and Allanore, A. (2019) 'Local fertilizers to achieve food self-sufficiency in Africa', *Science of the Total Environment*, 648, pp. 669–680. Available at: https://doi.org/10.1016/j.scitotenv.2018.08.154.

Cizauskas, C.A., Carlson, C.J., Burgio, K.R., Clements, C.F., Dougherty, E.R., Harris, N.C. and Phillips, A.J. (2017) 'Parasite vulnerability to climate change: an evidence-based functional trait approach', *Royal Society Open Science*, 4(1), p. 160535. Available at: https://doi.org/10.1098/rsos.160535.

Le Clec'h, W., Nordmeyer, S., Anderson, T.J.C. and Chevalier, F.D. (2022) 'Snails, microbiomes, and schistosomes: a three-way interaction?', *Trends in Parasitology*, 38(5), pp. 353–355. Available at: https://doi.org/10.1016/j.pt.2022.01.012.

Clerissi, C., Huot, C., Portet, A., Gourbal, B. and Toulza, E. (2023) 'Covariation between microeukaryotes and bacteria associated with Planorbidae snails', *PeerJ*. Edited by M. LaMontagne, 11, p. e16639. Available at: https://doi.org/10.7717/peerj.16639.

Cole, R.M., Richards, C.S. and Popkin, T.J. (1977) 'Novel bacterium infecting an African snail', *Journal of Bacteriology*, 132(3), pp. 950–966. Available at: https://doi.org/10.1128/jb.132.3.950-966.1977.

Collinge, S.K., Ray, C. and Cully, J.F. (2008) 'Effects of Disease on Keystone Species, Dominant Species, and their Communities', in R.S. Ostfeld, F. Keesing, and V.T. Eviner (eds) *Effects of Ecosystems on Disease and of Disease on Ecosystems*. Princeton: Princeton University Press, pp. 129–144. Available at: https://doi.org/10.1515/9781400837885.129.

Corno, G., Caravati, E., Callieri, C. and Bertoni, R. (2008) 'Effects of predation pressure on bacterial abundance, diversity, and size-structure distribution in an oligotrophic system', *Journal of Limnology*, 67(2), pp. 107–119. Available at: https://doi.org/10.4081/jlimnol.2008.107.

Dai, J.-R., Wang, W., Liang, Y.-S., Li, H.-J., Guan, X.-H. and Zhu, Y.-C. (2008) 'A novel molluscicidal formulation of niclosamide.', *Parasitology research*, 103(2), pp. 405–412. Available at: https://doi.org/10.1007/s00436-008-0988-2.

Dar, Y., Dreyfuss, G., Rondelaud, D. and Vignoles, P. (2015) 'Role of the lymnaeid snail Pseudosuccinea columella in the transmission of the liver fluke Fasciola hepatica in Egypt', *Journal of Helminthology*. 2014/05/27, 89(6), pp. 699–706. Available at: https://doi.org/DOI: 10.1017/S0022149X14000406.

Daskin, J.H. and Alford, R.A. (2012) 'Context-dependent symbioses and their potential roles in wildlife diseases', *Proceedings of the Royal Society B: Biological Sciences*, 279(1733), pp. 1457–1465. Available at: https://doi.org/10.1098/rspb.2011.2276.

Davoodi, S. and Foley, E. (2020) 'Host-Microbe-Pathogen Interactions: A Review of *Vibrio cholerae* Pathogenesis in *Drosophila*', *Frontiers in Immunology*, 10. Available at: https://doi.org/10.3389/fimmu.2019.03128.

Decaestecker, E., Gaba, S., Raeymaekers, J.A.M., Stoks, R., Van Kerckhoven, L., Ebert, D. and De Meester, L. (2007) 'Host-parasite "Red Queen" dynamics archived in pond sediment', *Nature*, 450(7171), pp. 870–873. Available at: https://doi.org/10.1038/nature06291.

Decaestecker, E., Van de Moortel, B., Mukherjee, S., Gurung, A., Stoks, R. and De Meester, L. (2024) 'Hierarchical eco-evo dynamics mediated by the gut microbiome', *Trends in Ecology and Evolution*, 39(2), pp. 165–174. Available at: https://doi.org/10.1016/j.tree.2023.09.013.

Decaestecker, E. and King, K. (2019) *Red Queen Dynamics*. 2nd edn, *Encyclopedia of Ecology: Volume 1-4, Second Edition*. 2nd edn. Elsevier Inc. Available at: https://doi.org/10.1016/B978-0-12-409548-9.10550-0.

Degeling, C., Johnson, J., Kerridge, I., Wilson, A., Ward, M., Stewart, C. and Gilbert, G. (2015) 'Implementing a One Health approach to emerging infectious disease: Reflections on the socio-political, ethical and legal dimensions', *BMC Public Health*, 15(1), pp. 1–11. Available at: https://doi.org/10.1186/s12889-015-2617-1.

Dell'Aglio, E. *et al.* (2023) 'Weevil Carbohydrate Intake Triggers Endosymbiont Proliferation: A Trade-Off between Host Benefit and Endosymbiont Burden', *mBio*, 14(2d), pp. e03333-22. Available at: https://doi.org/10.1128/mbio.03333-22.

Denev, S., Staykov, Y., Moutafchieva, R. and Beev, G. (2009) 'International Aquatic Research Microbial ecology of the gastrointestinal tract of fish and the potential application of probiotics and prebiotics in finfish aquaculture', *International Aquatic Research Int Aquat Res*, 1(1), pp. 1–29.

Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T.G. and Cryan, J.F. (2014) 'Microbiota is essential for

social development in the mouse', *Molecular Psychiatry*, 19(2), pp. 146–148. Available at: https://doi.org/10.1038/mp.2013.65.

Destoumieux-Garzón, D. *et al.* (2024) 'Cross-talk and mutual shaping between the immune system and the microbiota during an oyster's life', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 379(1901), p. 20230065. Available at: https://doi.org/10.1098/rstb.2023.0065.

Dheilly, N.M., Lucas, P. and Blanchard, Y. (2022) 'A World of Viruses Nested within Parasites: Unraveling Viral Diversity within Parasitic Flatworms (Platyhelminthes)', *Microbiology Spectrum*, 10(3), pp. 1–14. Available at: https://doi.org/10.1128/spectrum.00138-22.

Dheilly, N.M., Poulin, R. and Thomas, F. (2015) 'Biological warfare: Microorganisms as drivers of host-parasite interactions', *Infection, Genetics and Evolution*, 34(May), pp. 251–259. Available at: https://doi.org/10.1016/j.meegid.2015.05.027.

Dodson, B.L., Hughes, G.L., Paul, O., Matacchiero, A.C., Kramer, L.D. and Rasgon, J.L. (2014) '*Wolbachia* Enhances West Nile Virus (WNV) Infection in the Mosquito *Culex tarsalis*', *PLoS Neglected Tropical Diseases*, 8(7). Available at: https://doi.org/10.1371/journal.pntd.0002965.

Douchet, P., Boissier, J., Mulero, S., Ferté, H., Doberva, M., Allienne, J.-F., Toulza, E., Bethune, K. and Rey, O. (2022) 'Make visible the invisible: Optimized development of an environmental DNA metabarcoding tool for the characterization of trematode parasitic communities', *Environmental DNA*, 4(3), pp. 627–641. Available at: https://doi.org/https://doi.org/10.1002/edn3.273.

Douglas, A.E. (2009) 'The microbial dimension in insect nutritional ecology', *Functional Ecology*, 23(1), pp. 38–47. Available at: https://doi.org/10.1111/j.1365-2435.2008.01442.x.

Ducklow, H.W., Boyle, P.J., Maugel, P.W., Strong, C. and Mitchell, R. (1979) 'Bacterial flora of the schistosome vector snail *Biomphalaria glabrata*', *Applied and environmental microbiology*, 38(4), pp. 667–672. Available at: https://doi.org/10.1128/aem.38.4.667-672.1979.

Ducklow, H.W., Clausen, K. and Mitchell, R. (1981) 'Ecology of Bacterial Communities in the Schistosomiasis Vector Snail *Biomphalaria glabrata*', *Microbial Ecology*, 7(3), pp. 253–274. Available at: http://www.jstor.org/stable/4250667.

Dunlap, P. V, Ast, J.C., Kimura, S., Fukui, A. and Yoshino, T. (2007) 'Phylogenetic analysis of host–symbiont specificity and codivergencein bioluminescent symbioses', *Cladistics*, 23, pp. 507–532. Available at: https://doi.org/10.1111/j.1096-0031.2007.00157.x.

Duval, D. *et al.* (2015) 'A Novel Bacterial Pathogen of *Biomphalaria glabrata*: A Potential Weapon for Schistosomiasis Control?', *PLOS Neglected Tropical Diseases*, 9(2), p. e0003489. Available at: https://doi.org/10.1371/journal.pntd.0003489.

Erko, B., Abebe, F., Berhe, N., Medhin, G., Gebre-Michael, T., Gemetchu, T. and Gundersen, S.G. (2002) 'Control of Schistosoma mansoni by the soapberry endod (Phytolacca dodecandra) in Wollo, Northeastern ethiopia: Post-interventention prevalance', *East African Medical Journal*, 79, pp. 198–201.

Faro, M.J., Perazzini, M., Corrêa, L. dos R., Mello-Silva, C.C., Pinheiro, J., Mota, E.M., De Souza, S., De Andrade, Z. and Júnior, A.M. (2013) 'Biological, biochemical and histopathological features related to parasitic castration of Biomphalaria glabrata infected by Schistosoma mansoni', *Experimental Parasitology*, 134(2), pp. 228–234. Available at: https://doi.org/10.1016/j.exppara.2013.03.020.

Fashuyi, S.A. and Williams, M.O. (1977) 'The role of *Chaetogaster limnaei* in the dynamics of trematode transmission in natural populations of freshwater snails', *Zeitschrift für Parasitenkunde*,

54(1), pp. 55–60. Available at: https://doi.org/10.1007/BF00380636.

Faulwetter, S., Vasileiadou, A., Kouratoras, M., Dailianis, T. and Arvanitidis, C. (2013) 'Micro-computed tomography: Introducing new dimensions to taxonomy.', *ZooKeys*, (263), pp. 1–45. Available at: https://doi.org/10.3897/zookeys.263.4261.

Finlay, B.B. and McFadden, G. (2006) 'Anti-immunology: Evasion of the host immune system by bacterial and viral pathogens', *Cell*, 124(4), pp. 767–782. Available at: https://doi.org/10.1016/j.cell.2006.01.034.

Fischer, K., Tkach, V. V., Curtis, K.C. and Fischer, P.U. (2017) 'Ultrastructure and localization of *Neorickettsia* in adult digenean trematodes provides novel insights into helminth-endobacteria interaction', *Parasites and Vectors*, 10(1), pp. 1–15. Available at: https://doi.org/10.1186/s13071-017-2123-7.

Fleming-Davies, A.E., Williams, P.D., Dhondt, A.A., Dobson, A.P., Hochachka, W.M., Leon, A.E., Ley, D.H., Osnas, E.E. and Hawley, D.M. (2018) 'Incomplete host immunity favors the evolution of virulence in an emergent pathogen', *Science*, 359(6379), pp. 1030–1033. Available at: https://doi.org/10.1126/science.aao2140.

Fontaine, S.S. and Kohl, K.D. (2020) 'Gut microbiota of invasive bullfrog tadpoles responds more rapidly to temperature than a noninvasive congener', *Molecular Ecology*, 29(13), pp. 2449–2462. Available at: https://doi.org/https://doi.org/10.1111/mec.15487.

Foo, J.L., Ling, H., Lee, Y.S. and Chang, M.W. (2017) 'Microbiome engineering: Current applications and its future', *Biotechnology Journal*, 12(3), pp. 1–11. Available at: https://doi.org/10.1002/biot.201600099.

Ford, S.A. and King, K.C. (2016) 'Harnessing the Power of Defensive Microbes: Evolutionary Implications in Nature and Disease Control', *PLoS Pathogens*, 12(4), pp. 1–12. Available at: https://doi.org/10.1371/journal.ppat.1005465.

Forsyth, D.M. and Macdonald, G. (1965) 'Urological complications of endemic schistosomiasis in school-children', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 59(2), pp. 171–178.

French, E., Kaplan, I., Iyer-Pascuzzi, A., Nakatsu, C.H. and Enders, L. (2021) 'Emerging strategies for precision microbiome management in diverse agroecosystems', *Nature Plants*, 7(3), pp. 256–267. Available at: https://doi.org/10.1038/s41477-020-00830-9.

Galinier, R., Tetreau, G., Portet, A., Pinaud, S., Duval, D. and Gourbal, B. (2017) 'First characterization of viruses from freshwater snails of the genus *Biomphalaria*, the intermediate host of the parasite *Schistosoma mansoni*', *Acta Tropica*, 167, pp. 196–203. Available at: https://doi.org/https://doi.org/10.1016/j.actatropica.2016.12.021.

Gandon, S., Mackinnon, M.J., Nee, S. and Read, A.F. (2001) 'Imperfect vaccines and the evolution of pathogen virulence', *Nature*, 414(6865), pp. 751–756. Available at: https://doi.org/10.1038/414751a.

Gao, J., Yang, N., Lewis, F.A., Yau, P., Collins, J.J., Sweedler, J. V. and Newmark, P.A. (2019) 'A rotifer-derived paralytic compound prevents transmission of schistosomiasis to a mammalian host', *PLoS Biology*, 17(10), pp. 1–15. Available at: https://doi.org/10.1371/journal.pbio.3000485.

Geyer, K.K. *et al.* (2017) 'The *Biomphalaria glabrata* DNA methylation machinery displays spatial tissue expression, is differentially active in distinct snail populations and is modulated by interactions

with *Schistosoma mansoni*', *PLoS neglected tropical diseases*, 11(5), p. e0005246.

Godfray, H.C.J. *et al.* (2010) 'Food Security: The Challenge of Feeding 9 Billion People', *Science*, 327(5967), pp. 812–818. Available at: https://doi.org/10.1126/science.1185383.

Gomes, F.M. *et al.* (2017) 'Effect of naturally occurring *Wolbachia* in *Anopheles gambiae s.l.* mosquitoes from Mali on *Plasmodium falciparum* malaria transmission', *Proceedings of the National Academy of Sciences*, 114(47), pp. 12566–12571. Available at: https://doi.org/10.1073/pnas.1716181114.

Gómez, A. and Nichols, E. (2013) 'Neglected wild life: Parasitic biodiversity as a conservation target', *International Journal for Parasitology: Parasites and Wildlife*, 2(1), pp. 222–227. Available at: https://doi.org/10.1016/j.ijppaw.2013.07.002.

Gould, A.L. *et al.* (2018) 'Microbiome interactions shape host fitness', *Proceedings of the National Academy of Sciences of the United States of America*, 115(51), pp. E11951–E11960. Available at: https://doi.org/10.1073/pnas.1809349115.

Gower, C.M., Vince, L. and Webster, J.P. (2017) 'Should we be treating animal schistosomiasis in Africa? The need for a One Health economic evaluation of schistosomiasis control in people and their livestock', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 111(6), pp. 244–247. Available at: https://doi.org/10.1093/trstmh/trx047.

Grabner, D.S., Mohamed, F.A.M.M., Nachev, M., Me, E.M.H., Sabry, A.H.A., Sures, B., Méabed, E.M.H., Sabry, A.H.A. and Sures, B. (2014) 'Invasion biology meets parasitology: A case study of parasite spill-back with egyptian *Fasciola gigantica* in the invasive snail *Pseudosuccinea columella*', *PLoS ONE*, 9(2), pp. 1–7. Available at: https://doi.org/10.1371/journal.pone.0088537.

Graça, A.P., Bondoso, J., Gaspar, H., Xavier, J.R., Monteiro, M.C., de la Cruz, M., Oves-Costales, D., Vicente, F. and Lage, O.M. (2013) 'Antimicrobial Activity of Heterotrophic Bacterial Communities from the Marine Sponge *Erylus discophorus* (Astrophorida, Geodiidae)', *PLOS ONE*, 8(11), p. e78992.

Greenspan, S.E. *et al.* (2019) 'Arthropod-bacteria interactions influence assembly of aquatic host microbiome and pathogen defense', *Proceedings of the Royal Society B: Biological Sciences*, 286(1905). Available at: https://doi.org/10.1098/rspb.2019.0924.

Greyson-Gaito, C.J., Bartley, T.J., Cottenie, K., Jarvis, W.M.C., Newman, A.E.M. and Stothart, M.R. (2020) 'Into the wild: microbiome transplant studies need broader ecological reality', *Proceedings of the Royal Society B: Biological Sciences*, 287(1921), p. 20192834. Available at: https://doi.org/10.1098/rspb.2019.2834.

Grizzle, J.M. and Brunner, C.J. (2009) 'Infectious Diseases of Freshwater Mussels and Other Freshwater Bivalve Mollusks', *Reviews in Fisheries Science*, 17(4), pp. 425–467. Available at: https://doi.org/10.1080/10641260902879000.

Gutiérrez, A., Pointier, J.-P., Yong, M., Sánchez, J. and Théron, A. (2003) 'Evidence of phenotypic differences between resistant and susceptible isolates of Pseudosuccinea columella (Gastropoda: Lymnaeidae) to Fasciola hepatica (Trematoda: Digenea) in Cuba', *Parasitology Research*, 90(2), pp. 129–134. Available at: https://doi.org/10.1007/s00436-002-0824-z.

Haas, W., Grabe, K., Geis, C., Päch, T., Stoll, K., Fuchs, M., Haberl, B. and Loy, C. (2002) 'Recognition and invasion of human skin by Schistosoma mansoni cercariae: The key-role of L-arginine', *Parasitology*, 124(2), pp. 153–167. Available at: https://doi.org/10.1017/S0031182001001032.

Hahn, M.A., Rosario, K., Lucas, P. and Dheilly, N.M. (2020) 'Characterization of viruses in a

tapeworm: phylogenetic position, vertical transmission, and transmission to the parasitized host', *ISME Journal*, 14(7), pp. 1755–1767. Available at: https://doi.org/10.1038/s41396-020-0642-2.

Halstead, N.T. *et al.* (2018) 'Agrochemicals increase risk of human schistosomiasis by supporting higher densities of intermediate hosts', *Nature Communications*, 9(837), pp. 1–10. Available at: https://doi.org/10.1038/s41467-018-03189-w.

Hammer, T.J., Janzen, D.H., Hallwachs, W., Jaffe, S.P. and Fierer, N. (2017) 'Caterpillars lack a resident gut microbiome', *Proceedings of the National Academy of Sciences of the United States of America*, 114(36), pp. 9641–9646. Available at: https://doi.org/10.1073/pnas.1707186114.

Hammoud, C. *et al.* (2022) 'Simultaneous genotyping of snails and infecting trematode parasites using high-throughput amplicon sequencing', *Molecular Ecology Resources*, 22(2), pp. 567–586. Available at: https://doi.org/10.1111/1755-0998.13492.

Harris, R.N. *et al.* (2009) 'Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus', *The ISME Journal*, 3(7), pp. 818–824. Available at: https://doi.org/10.1038/ismej.2009.27.

Harrison, X.A., Price, S.J., Hopkins, K., Leung, W.T.M., Sergeant, C. and Garner, T.W.J. (2019) 'Diversity-Stability Dynamics of the Amphibian Skin Microbiome and Susceptibility to a Lethal Viral Pathogen', *Frontiers in Microbiology*, 10. Available at: https://doi.org/10.3389/fmicb.2019.02883.

Havel, J.E., Lee, C.E. and Vander Zander, M.J. (2005) 'Do Reservoirs Facilitate Invasions into Landscapes?', *BioScience*, 55(6), pp. 518–525. Available at: https://doi.org/10.1641/0006-3568(2005)055[0518:DRFIIL]2.0.CO;2.

He, S., Wang, Q., Li, S., Ran, C., Guo, X., Zhang, Z. and Zhou, Z. (2017) 'Antibiotic growth promoter olaquindox increases pathogen susceptibility in fish by inducing gut microbiota dysbiosis', *Science China Life Sciences*, 60(11), pp. 1260–1270. Available at: https://doi.org/10.1007/s11427-016-9072-6.

Heard, M.J., Smith, K.F., Ripp, K., Berger, M., Chen, J., Dittmeier, J., Goter, M., Mcgarvey, S.T. and Ryan, E. (2013) 'Increased threat of disease as species move towards extinction', *Conservation Biology*, 27(6), pp. 1378–1388. Available at: https://doi.org/10.1111/cobi.12143.Increased.

Hechinger, R.F. (2023) 'Let's restart formally naming "larval" trematodes', *Trends in Parasitology*, 39(8), pp. 638–649. Available at: https://doi.org/https://doi.org/10.1016/j.pt.2023.05.011.

Heeren, S. *et al.* (2023) 'Diversity and dissemination of viruses in pathogenic protozoa', *Nature Communications*, 14(1). Available at: https://doi.org/10.1038/s41467-023-44085-2.

Henrich, T., Benesh, D.P. and Kalbe, M. (2013) 'Hybridization between two cestode species and its consequences for intermediate host range', *Parasites and Vectors*, 6(1), pp. 1–9. Available at: https://doi.org/10.1186/1756-3305-6-33.

Henry, L.P., Bruijning, M., Forsberg, S.K.G. and Ayroles, J.F. (2021) 'The microbiome extends host evolutionary potential', *Nature Communications*, 12(1), pp. 1–13. Available at: https://doi.org/10.1038/s41467-021-25315-x.

Hertel, J., Holweg, A., Haberl, B., Kalbe, M. and Haas, W. (2006) 'Snail odour-clouds: Spreading and contribution to the transmission success of *Trichobilharzia ocellata* (Trematoda, Digenea) miracidia', *Oecologia*, 147(1), pp. 173–180. Available at: https://doi.org/10.1007/s00442-005-0239-5.

Hertel, L.A., Barbosa, C.S., Santos, R.A.A.L. and Loker, E.S. (2004) 'Molecular identification of

symbionts from the pulmonate snail *Biomphalaria glabrata* in Brazil', *Journal of Parasitology*, 90(4), pp. 759–763. Available at: https://doi.org/10.1645/GE-223R.

Hoa Le, T., Van De, N., Agatsuma, T., Giang Thi Nguyen, T., Doanh Nguyen, Q., McManus, D.P. and Blair, D. (2008) 'Human fascioliasis and the presence of hybrid/introgressed forms of *Fasciola hepatica* and *Fasciola gigantica* in Vietnam', *International Journal for Parasitology*, 38, pp. 725–730. Available at: https://doi.org/10.1016/j.ijpara.2007.10.003.

Hoffmann, A.A. *et al.* (2011) 'Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission.', *Nature*, 476(7361), pp. 454–457. Available at: https://doi.org/10.1038/nature10356.

Hoover, C.M., Rumschlag, S.L., Strgar, L., Arakala, A., Gambhir, M., de Leo, G.A., Sokolow, S.H., Rohr, J.R. and Remais, J. V (2020) 'Effects of agrochemical pollution on schistosomiasis transmission: a systematic review and modelling analysis.', *The Lancet. Planetary health*, 4(7), pp. e280–e291. Available at: https://doi.org/10.1016/S2542-5196(20)30105-4.

Horák, P., Mikeš, L., Panská, L., Skála, V., Soldánová, M. and Brant, S. (2015) 'Avian Schistosomes and Outbreaks of Cercarial Dermatitis', *Clinical microbiology reviews*, 28(1), pp. 165–190. Available at: https://doi.org/10.1128/CMR.00043-14.

Houwenhuyse, S., Stoks, R., Mukherjee, S. and Decaestecker, E. (2021) 'Locally adapted gut microbiomes mediate host stress tolerance', *The ISME Journal*, 15(8), pp. 2401–2414. Available at: https://doi.org/10.1038/s41396-021-00940-y.

Howarth, F.G. (1991) 'Environmental Impacts of Classical Biological Control', *Annual Review of Entomology*, 36(1), pp. 485–509. Available at: https://doi.org/10.1146/annurev.en.36.010191.002413.

Hu, Z., Tong, Q., Chang, J., Yu, J., Li, S., Niu, H. and Ma, D. (2021) 'Gut bacterial communities in the freshwater snail Planorbella trivolvis and their modification by a non-herbivorous diet', *PeerJ*. Edited by J. Gillespie, 9, p. e10716. Available at: https://doi.org/10.7717/peerj.10716.

Hughes, G.L., Vega-Rodriguez, J., Xue, P. and Rasgon, J.L. (2012) '*Wolbachia* strain wAlbB enhances infection by the rodent malaria parasite *Plasmodium berghei* in *Anopheles gambiae* mosquitoes', *Applied and Environmental Microbiology*, 78(5), pp. 1491–1495. Available at: https://doi.org/10.1128/AEM.06751-11.

Hulme, P.E. (2009) 'Trade, transport and trouble: managing invasive species pathways in an era of globalization', *Journal of Applied Ecology*, 46, pp. 10–18. Available at: https://doi.org/10.1111/j.1365-2664.2008.01600.x.

Huot, C., Clerissi, C., Gourbal, B., Galinier, R., Duval, D. and Toulza, E. (2020) 'Schistosomiasis Vector Snails and Their Microbiota Display a Phylosymbiosis Pattern', *Frontiers in Microbiology*, 10(January), pp. 1–10. Available at: https://doi.org/10.3389/fmicb.2019.03092.

Hussain, M., Frentiu, F.D., Moreira, L.A., O'Neill, S.L. and Asgari, S. (2011) '*Wolbachia* uses host microRNAs to manipulate host gene expression and facilitate colonization of the dengue vector *Aedes aegypti*', *Proceedings of the National Academy of Sciences of the United States of America*, 108(22), pp. 9250–9255. Available at: https://doi.org/10.1073/pnas.1105469108.

Huyse, T., Webster, B.L., Geldof, S., Stothard, J.R., Diaw, O.T. and Rollinson, D. (2009) 'Bidirectional Introgressive Hybridization between a Cattle and Human Schistosome Species', *PLoS PATHOGENS*, 5(9). Available at: https://doi.org/10.1371/journal.ppat.1000571.

Ip, K.K.L., Liang, Y., Lin, L., Wu, H., Xue, J. and Qiu, J.-W. (2014) 'Biological control of invasive apple snails by two species of carp: Effects on non-target species matter', *Biological Control*, 71, pp. 16–22. Available at: https://doi.org/https://doi.org/10.1016/j.biocontrol.2013.12.009.

Ittiprasert, W. and Knight, M. (2012) 'Reversing the resistance phenotype of the biomphalaria glabrata snail host schistosoma mansoni infection by temperature modulation', *PLoS Pathogens*, 8(4). Available at: https://doi.org/10.1371/journal.ppat.1002677.

Ives, A. *et al.* (2011) '*Leishmania* RNA Virus Controls the Severity of Mucocutaneous Leishmaniasis', *Science*, 331(11), pp. 775–778. Available at: https://doi.org/10.1126/science.1199326.

Jahn, M.T., Markert, S.M., Ryu, T., Ravasi, T., Stigloher, C., Hentschel, U. and Moitinho-Silva, L. (2016) 'Shedding light on cell compartmentation in the candidate phylum Poribacteria by high resolution visualisation and transcriptional profiling', *Scientific Reports*, 6(May), pp. 1–9. Available at: https://doi.org/10.1038/srep35860.

Jani, A.J., Bushell, J., Arisdakessian, C.G., Belcaid, M., Boiano, D.M., Brown, C. and Knapp, R.A. (2021) 'The amphibian microbiome exhibits poor resilience following pathogen-induced disturbance', *ISME Journal*, 15(6), pp. 1628–1640. Available at: https://doi.org/10.1038/s41396-020-00875-w.

Johnson, J.S. *et al.* (2019) 'Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis', *Nature Communications*, 10(1), pp. 1–11. Available at: https://doi.org/10.1038/s41467-019-13036-1.

Jokela, J., Dybdahl, M.F. and Lively, C.M. (2009) 'The maintenance of sex, clonal dynamics, and host-parasite coevolution in a mixed population of sexual and asexual snails', *American Naturalist*, 174. Available at: https://doi.org/10.1086/599080.

Jones, R.A., Brophy, P.M., Mitchell, E.S. and Williams, H.W. (2017) 'Rumen fluke (*Calicophoron daubneyi*) on Welsh farms: Prevalence, risk factors and observations on co-infection with *Fasciola hepatica*', *Parasitology*, 144(2), pp. 237–247. Available at: https://doi.org/10.1017/S0031182016001797.

De Jong-Brink, M., Bergamin-Sassen, M. and Soto, M.S. (2001) 'Multiple strategies of schistosomes to meet their requirements in the intermediate snail host', *Parasitology*, 123, pp. 129–141. Available at: https://doi.org/10.1017/s0031182001008149.

Jorge, F., Dheilly, N.M., Froissard, C. and Poulin, R. (2022a) 'Association between parasite microbiomes and caste development and colony structure in a social trematode', *Molecular Ecology*, 31(21), pp. 5608–5617. Available at: https://doi.org/10.1111/mec.16671.

Jorge, F., Froissard, C., Dheilly, N.M. and Poulin, R. (2022b) 'Bacterial community dynamics following antibiotic exposure in a trematode parasite', *International Journal for Parasitology*, 52(5), pp. 265–274. Available at: https://doi.org/10.1016/j.ijpara.2021.11.006.

Jorge, F., Dheilly, N.M. and Poulin, R. (2020) 'Persistence of a Core Microbiome Through the Ontogeny of a Multi-Host Parasite', *Frontiers in Microbiology*, 11(May). Available at: https://doi.org/10.3389/fmicb.2020.00954.

Kalbe, M., Haberl, B. and Haas, W. (1997) 'Miracidial host-finding in *Fasciola hepatica* and *Trichobilharzia ocellata* is stimulated by species-specific glycoconjugates released from the host snails', *Parasitology Research*, 83(8), pp. 806–812. Available at: https://doi.org/10.1007/s004360050344.

Kaparakis-Liaskos, M. and Ferrero, R.L. (2015) 'Immune modulation by bacterial outer membrane

vesicles', *Nature Reviews Immunology*, 15(6), pp. 375–387. Available at: https://doi.org/10.1038/nri3837.

Karvonen, A., Hudson, P.J., Seppälä, O. and Valtonen, E.T. (2004) 'Transmission dynamics of a trematode parasite: Exposure, acquired resistance and parasite aggregation', *Parasitology Research*, 92(3), pp. 183–188. Available at: https://doi.org/10.1007/s00436-003-1035-y.

Kelly, D.W., Paterson, R.A., Townsend, C.R., Poulin, R. and Tompkins, D.M. (2009) 'Parasite spillback: A neglected concept in invasion ecology?', *Ecology*, 90(8), pp. 2047–2056. Available at: https://doi.org/https://doi.org/10.1890/08-1085.1.

Kimura, K. and Tomaru, Y. (2014) 'Coculture with marine bacteria confers resistance to complete viral lysis of diatom cultures', *Aquatic Microbial Ecology*, 73(1), pp. 69–80. Available at: https://doi.org/10.3354/ame01705.

King, C.H. and Dangerfield-Cha, M. (2008) 'The unacknowledged impact of chronic schistosomiasis', *Chronic Illness*, 4, pp. 65–79. Available at: https://doi.org/10.1177/1742395307084407.

King, K.C., Jokela, J. and Lively, C.M. (2011) 'Trematode parasites infect or die in snail hosts', *Biology Letters*, 7(2), pp. 265–268. Available at: https://doi.org/10.1098/rsbl.2010.0857.

Knight, M. *et al.* (2015) 'Susceptibility of Snails to Infection with Schistosomes is influenced by Temperature and Expression of Heat Shock Proteins.', *Epidemiology*, 5(2), p. 189. Available at: https://doi.org/10.4172/2161-1165.1000189.

Knight, M., Ittiprasert, W., Arican-Goktas, H.D. and Bridger, J.M. (2016) 'Epigenetic modulation, stress and plasticity in susceptibility of the snail host, *Biomphalaria glabrata*, to *Schistosoma mansoni* infection', *International Journal for Parasitology*, 46(7), pp. 389–394. Available at: https://doi.org/https://doi.org/10.1016/j.ijpara.2016.03.003.

Knight, R. *et al.* (2018) 'Best practices for analysing microbiomes', *Nature Reviews Microbiology*, 16(7), pp. 410–422. Available at: https://doi.org/10.1038/s41579-018-0029-9.

Knutie, S.A., Wilkinson, C.L., Kohl, K.D. and Rohr, J.R. (2017) 'Early-life disruption of amphibian microbiota decreases later-life resistance to parasites', *Nature Communications*, 8(1), pp. 1–8. Available at: https://doi.org/10.1038/s41467-017-00119-0.

Koch, H. and Schmid-Hempel, P. (2012) 'Gut microbiota instead of host genotype drive the specificity in the interaction of a natural host-parasite system', *Ecology Letters*, 15(10), pp. 1095–1103. Available at: https://doi.org/10.1111/j.1461-0248.2012.01831.x.

Kokaliaris, C. *et al.* (2022) 'Effect of preventive chemotherapy with praziquantel on schistosomiasis among school-aged children in sub-Saharan Africa: a spatiotemporal modelling study', *The Lancet Infectious Diseases*, 22(1), pp. 136–149. Available at: https://doi.org/10.1016/S1473-3099(21)00090-6.

Krotman, Y., Yergaliyev, T.M., Alexander Shani, R., Avrahami, Y. and Szitenberg, A. (2020) 'Dissecting the factors shaping fish skin microbiomes in a heterogeneous inland water system', *Microbiome*, 8(1), p. 9. Available at: https://doi.org/10.1186/s40168-020-0784-5.

Krull, W.H. (1933) 'The snail Pseudosuccinea columella (Say) as a potentially important intermediate host in extending the range of Fasciola hepatica Linn', *Journal of the Washington Academy of Sciences*, 23(8), pp. 389–391. Available at: http://www.jstor.org/stable/24530351.

Kuris, A.M. *et al.* (2008) 'Ecosystem energetic implications of parasite and free-living biomass in

three estuaries', *Nature*, 454(7203), pp. 515–518. Available at: https://doi.org/10.1038/nature06970.

Kwak, M.L., Heath, A.C.G. and Cardoso, P. (2020) 'Methods for the assessment and conservation of threatened animal parasites', *Biological Conservation*, 248, p. 108696. Available at: https://doi.org/https://doi.org/10.1016/j.biocon.2020.108696.

Lafferty, K.D., Dobson, A.P. and Kuris, A.M. (2006) 'Parasites dominate food web links', *Proceedings of the National Academy of Sciences*, 103(30), pp. 11211–11216. Available at: https://doi.org/10.1073/pnas.0604755103.

Lafferty, K.D. and Kuris, A.M. (2005) 'Parasitism and environmental disturbances', in F. Thomas, F. Renaud, and J.-F. Guegan (eds) *Parasitism and Ecosystems*. Oxford University Press, pp. 113–123. Available at: https://doi.org/10.1093/acprof:oso/9780198529873.003.0008.

Lai, P.F. and Canning, E.U. (1980) 'Infectivity of a microsporidium of mosquitoes (Nosema algerae) to larval stages of Schistosoma mansoni in Biomphalaria glabrata', *International Journal for Parasitology*, 10(4), pp. 293–301. Available at: https://doi.org/https://doi.org/10.1016/0020-7519(80)90010-7.

Laidemitt, M.R., Anderson, L.C., Wearing, H.J., Mutuku, M.W., Mkoji, G.M. and Loker, E.S. (2019) 'Antagonism between parasites within snail hosts impacts the transmission of human schistosomiasis', *eLife*, 8. Available at: https://doi.org/10.7554/eLife.50095.

Landmann, F., Voronin, D., Sullivan, W. and Taylor, M.J. (2011) 'Anti-filarial activity of antibiotic therapy is due to extensive apoptosis after Wolbachia depletion from filarial nematodes', *PLoS Pathogens*, 7(11), pp. 1–11. Available at: https://doi.org/10.1371/journal.ppat.1002351.

Lee, J., Venna, V.R., Durgan, D.J., Shi, H., Hudobenko, J., Putluri, N., Petrosino, J., McCullough, L.D. and Bryan, R.M. (2020) 'Young versus aged microbiota transplants to germ-free mice: increased short-chain fatty acids and improved cognitive performance.', *Gut microbes*, 12(1), pp. 1–14. Available at: https://doi.org/10.1080/19490976.2020.1814107.

Lee, Y.K. and Mazmanian, S.K. (2010) 'Has the Microbiota Played a Critical Role in the Evolution of the Adaptive Immune System?', *Science*, 330(6012), pp. 1768–1773. Available at: https://doi.org/10.1126/science.1195568.

Leger, E. and Webster, J.P. (2017) 'Hybridizations within the Genus Schistosoma: Implications for evolution, epidemiology and control', *Parasitology*. Available at: https://doi.org/10.1017/S0031182016001190.

Leicht, K., Jokela, J. and Seppälä, O. (2013) 'An experimental heat wave changes immune defense and life history traits in a freshwater snail', *Ecology and Evolution*, 3(15), pp. 4861–4871. Available at: https://doi.org/10.1002/ece3.874.

Lemma, A. (1970) 'Laboratory and field evaluation of the molluscicidal properties of Phytolacca dodecandra.', *Bulletin of the World Health Organization*, 42(4), pp. 597–612.

De Leo, G.A., Stensgaard, A.S., Sokolow, S.H., N'Goran, E.K., Chamberlin, A.J., Yang, G.J., Utzinger, J. and De Leo, G.A. (2020) 'Schistosomiasis and climate change', *The BMJ*, 371(November), pp. 1–8. Available at: https://doi.org/10.1136/bmj.m4324.

Levy, R. and Borenstein, E. (2014) 'Metagenomic systems biology and metabolic modeling of the human microbiome', *Gut Microbes*, 5(2), pp. 265–270. Available at: https://doi.org/10.4161/gmic.28261.

Li, L.-H. *et al.* (2019) 'Spatial structure of the microbiome in the gut of *Pomacea canaliculata*', *BMC Microbiology*, 19(1), p. 273. Available at: https://doi.org/10.1186/s12866-019-1661-x.

Lim, H.K. and Heyneman, D. (1972) 'Intramolluscan inter-trematode antagonism: a review of factors influencing the host-parasite system and its possible role in biological control.', *Advances in parasitology*, 10, pp. 191–268. Available at: https://doi.org/10.1016/s0065-308x(08)60175-x.

Lin, D., Hong, J., Sanogo, B., Du, S., Xiang, S., Hui, J.H.-L., Ding, T., Wu, Z. and Sun, X. (2023) 'Core gut microbes *Cloacibacterium* and *Aeromonas* associated with different gastropod species could be persistently transmitted across multiple generations', *Microbiome*, 11(1), p. 267. Available at: https://doi.org/10.1186/s40168-023-01700-0.

Littlewood, D.T.J., Rohde, K. and Clough, K.A. (1999) 'The interrelationships of all major groups of Platyhelminthes: phylogenetic evidence from morphology and molecules', *Biological Journal of the Linnean Society*, 66(1), pp. 75–114. Available at: https://doi.org/10.1111/j.1095-8312.1999.tb01918.x.

Liu, M., Sui, C., Zhao, W., Fan, C., Zhang, Y., Qiu, Z., Wang, Y., Zhang, Q. and Liu, Y. (2025) 'Dynamic response of gut microbiota mediates the adaptation of *Cipangopaludina chinensis* to *Pomacea canaliculata* invasion', *Microbiome*, 13(1), p. 171. Available at: https://doi.org/10.1186/s40168-025-02160-4.

Lockyer, A.E. *et al.* (2012) 'Early Differential Gene Expression in Haemocytes from Resistant and Susceptible Biomphalaria glabrata Strains in Response to Schistosoma mansoni', *PLOS ONE*, 7(12), p. e51102.

Loker, E.S., Dejong, R.J. and Brant, S. V. (2022) 'Scratching the Itch: Updated Perspectives on the Schistosomes Responsible for Swimmer's Itch around the World', *Pathogens*, 11(5), pp. 1–27. Available at: https://doi.org/10.3390/pathogens11050587.

Louhi, K.R., Sundberg, L.R., Jokela, J. and Karvonen, A. (2015) 'Interactions among bacterial strains and fluke genotypes shape virulence of co-infection', *Proceedings of the Royal Society B: Biological Sciences*, 282(1821). Available at: https://doi.org/10.1098/rspb.2015.2097.

Lounnas, M. *et al.* (2017) 'Self-fertilization, long-distance flash invasion and biogeography shape the population structure of *Pseudosuccinea columella* at the worldwide scale', *Molecular Ecology*, 26(3), pp. 887–903. Available at: https://doi.org/10.1111/mec.13984.

Lydeard, C. *et al.* (2004) 'The Global Decline of Nonmarine Mollusks', *BioScience*, 54(4), pp. 321–330. Available at: https://doi.org/10.1641/0006-3568(2004)054[0321:TGDONM]2.0.CO;2.

Macke, E., Callens, M., De Meester, L. and Decaestecker, E. (2017a) 'Host-genotype dependent gut microbiota drives zooplankton tolerance to toxic cyanobacteria', *Nature Communications*, 8(1), p. 1608. Available at: https://doi.org/10.1038/s41467-017-01714-x.

Macke, E., Tasiemski, A., Massol, F., Callens, M. and Decaestecker, E. (2017b) 'Life history and eco-evolutionary dynamics in light of the gut microbiota', *Oikos*, 126(4), pp. 508–531. Available at: https://doi.org/10.1111/oik.03900.

Maes, T. (2024) *Snails in a changing world – Potential for local adaptation and distribution changes in an intermediate host of schistosomiasis*.

Magalhães, L.A., Zanotti-Magalhães, E.M. and de Carvalho, J.F. (1997) 'Observations on the miraxonal attraction exercised by sexually immature or adult *Biomphalaria glabrata* infected or not by *Schistosoma mansoni*.', *Revista de saude publica*, 31(2), pp. 121–124. Available at:

https://doi.org/10.1590/s0034-89101997000200003.

Mahmoud, A.A.F. (2001) *Schistosomiasis*. volume 3. Edited by G. Pasvol and S. Hoffman. Tropical Medicine Science and Practice.

Mallon, C.A., Elsas, J.D. van and Salles, J.F. (2015) 'Microbial Invasions: The Process, Patterns, and Mechanisms', *Trends in Microbiology*, 23(11), pp. 719–729. Available at: https://doi.org/10.1016/j.tim.2015.07.013.

Manoj, R.R.S., Latrofa, M.S., Epis, S. and Otranto, D. (2021) 'Wolbachia: endosymbiont of onchocercid nematodes and their vectors', *Parasites and Vectors*, 14(1), pp. 1–24. Available at: https://doi.org/10.1186/s13071-021-04742-1.

Margulis, L. (1991) 'Symbiogenesis and symbionticism', in M. Lynn and R. Fester (eds) *Symbiosis as a source of evolutionary innovation: speciation and morphogenesis*. Cambridge: MIT press, pp. 1–14.

Mas-Coma, S., Valero, M.A. and Bargues, M.D. (2009a) 'Chapter 2 Fasciola, Lymnaeids and Human Fascioliasis, with a Global Overview on Disease Transmission, Epidemiology, Evolutionary Genetics, Molecular Epidemiology and Control', *Advances in Parasitology*, 69(09), pp. 41–146. Available at: https://doi.org/10.1016/S0065-308X(09)69002-3.

Mas-Coma, S., Valero, M.A. and Bargues, M.D. (2009b) 'Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis.', *Veterinary parasitology*, 163(4), pp. 264–280. Available at: https://doi.org/10.1016/j.vetpar.2009.03.024.

Mazuecos, L. *et al.* (2023) 'Frankenbacteriosis targeting interactions between pathogen and symbiont to control infection in the tick vector', *iScience*, 26(5). Available at: https://doi.org/10.1016/j.isci.2023.106697.

McAllister, D.E., Craig, J., Davidson, N., Delany, S. and Seddon, M. (2001) *Biodiversity Impacts of Large Dams*.

McCann, P., McFarland, C., Megaw, J., Siu-Ting, K., Cantacessi, C., Rinaldi, G. and Gobert, G.N. (2024) 'Assessing the microbiota of the snail intermediate host of trematodes, *Galba truncatula*', *Parasites and Vectors*, 17(1), pp. 1–16. Available at: https://doi.org/10.1186/s13071-024-06118-7.

McCully, R.M., van Niekerk, J.W. and Kruger, S.P. (1967) 'Observations on the pathology of bilharziasis and other parasitic infestations of *Hippopotamus amphibius* Linnaeus, 1758, from the Kruger National Park', *Onderstepoort Journal of Veterinary Research*, 34(2), pp. 563–618.

McLaren, M.R. and Callahan, B.J. (2020) 'Pathogen resistance may be the principal evolutionary advantage provided by the microbiome', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375(1808), p. 20190592. Available at: https://doi.org/10.1098/rstb.2019.0592.

McMaster, R.W., Morrison, C.J. and Kobor, M.S. (2016) 'Epigenetics: A New Model for Intracellular Parasite-Host Cell Regulation', *Trends in Parasitology*, 32(7), pp. 515–521. Available at: https://doi.org/10.1016/j.pt.2016.04.002.

McMeniman, C.J. and O'Neill, S.L. (2010) 'A Virulent Wolbachia infection decreases the viability of the dengue vector Aedes aegypti during periods of embryonic quiescence', *PLoS Neglected Tropical Diseases*, 4(7), p. e748. Available at: https://doi.org/10.1371/journal.pntd.0000748.

Mehmood, K. *et al.* (2017) 'A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants', *Microbial Pathogenesis*, 109, pp. 253–262. Available at: https://doi.org/10.1016/j.micpath.2017.06.006.

Meng, Y., Sun, J. and Zhang, G. (2023) 'Fecal microbiota transplantation holds the secret to youth', *Mechanisms of Ageing and Development*, 212, p. 111823. Available at: https://doi.org/https://doi.org/10.1016/j.mad.2023.111823.

Meurs, L., Mbow, M., Vereecken, K., Menten, J., Mboup, S. and Polman, K. (2012) 'Epidemiology of mixed Schistosoma mansoni and Schistosoma haematobium infections in northern Senegal', *International Journal for Parasitology*, 42(3), pp. 305–311. Available at: https://doi.org/10.1016/j.ijpara.2012.02.002.

Milotic, M., Lymbery, A., Thompson, A., Doherty, J.-F. and Godfrey, S. (2020) 'Parasites are endangered by the conservation of their hosts: Meta-analyses of the effect of host captivity on the odds of parasite infection', *Biological Conservation*, 248, p. 108702. Available at: https://doi.org/https://doi.org/10.1016/j.biocon.2020.108702.

Molehin, A.J. (2020) 'Schistosomiasis vaccine development: Update on human clinical trials', *Journal of Biomedical Science*, 27(1), pp. 1–7. Available at: https://doi.org/10.1186/s12929-020-0621-y.

Moreira, L.A. *et al.* (2009) 'A Wolbachia Symbiont in *Aedes aegypti* Limits Infection with Dengue, Chikungunya, and Plasmodium', *Cell*, 139(7), pp. 1268–1278. Available at: https://doi.org/10.1016/J.CELL.2009.11.042.

Morgan, J.A.T. *et al.* (2003) 'First Report of a Natural Hybrid Between *Schistosoma mansoni* and *S. rodhaini*', *Journal of Parasitology*, 89(2), pp. 416–418. Available at: https://doi.org/10.1645/0022-3395(2003)089[0416:FROANH]2.0.CO;2.

Morris, G.P. (1973) 'The morphology of associations between a trematode (*Megalodiscus temperatus*) and bacteria', *Canadian Journal of Zoology*, 51(12), pp. 1313–1314. Available at: https://doi.org/10.1139/z73-186.

Mosca, A., Leclerc, M. and Hugot, J.P. (2016) 'Gut Microbiota Diversity and Human Diseases: Should We Reintroduce Key Predators in Our Ecosystem?', *Frontiers in Microbiology*, 7.

Mudavanhu, A., Schols, R., Goossens, E., Nhiwatiwa, T., Manyangadze, T., Brendonck, L. and Huyse, T. (2024) 'One Health monitoring reveals invasive freshwater snail species, new records, and undescribed parasite diversity in Zimbabwe', *Parasites and Vectors*, 17(1), pp. 1–17. Available at: https://doi.org/10.1186/s13071-024-06307-4.

Mueller, U.G. and Sachs, J.L. (2015) 'Engineering Microbiomes to Improve Plant and Animal Health', *Trends in Microbiology*, 23(10), pp. 606–617. Available at: https://doi.org/10.1016/j.tim.2015.07.009.

Mukaratirwa, S., Kristensen, T.K., Siegismund, H.R. and Chandiwana, S.K. (1998) 'Genetic and morphological variation of populations belonging to the Bulinus truncatus/tropicus complex (Gastropoda: Planorbidae) in South Western Zimbabwe.', *Journal of molluscan studies*, 64, pp. 435–446.

Mulero, S., Rey, O., Arancibia, N., Mas-Coma, S. and Boissier, J. (2019) 'Persistent establishment of a tropical disease in Europe: the preadaptation of schistosomes to overwinter', *Parasites & Vectors*, 12(1), p. 379. Available at: https://doi.org/10.1186/s13071-019-3635-0.

Mutapi, F., Maizels, R., Fenwick, A. and Woolhouse, M. (2017) 'Human schistosomiasis in the post mass drug administration era', *The Lancet Infectious Diseases*, 17(2), pp. 1–7. Available at: https://doi.org/10.1016/S1473-3099(16)30475-3.

Nacif-Pimenta, R., Mattos, A.C.A. de, Orfanó, A. da S., Barbosa, L., Pimenta, P.F.P. and Coelho, P.M.Z.

(2012) '*Schistosoma mansoni* in Susceptible and Resistant Snail Strains *Biomphalaria tenagophila*: In Vivo Tissue Response and In Vitro Hemocyte Interactions', *PLOS ONE*, 7(9), p. e45637.

Nguyen, T.B.N., De, N. Van, Nguyen, T.K.L., Quang, H.H., Doan, H.T.T., Agatsuma, T. and Le, T.H. (2018) 'Distribution Status of Hybrid Types in Large Liver Flukes, Fasciola Species (Digenea: Fasciolidae), from Ruminants and Humans in Vietnam', *The Korean journal of parasitology*, 56(5), pp. 453–461. Available at: https://doi.org/10.3347/kjp.2018.56.5.453.

Núñez, P.E., Adema, C.M. and De Jong-Brink, M.D. (1994) 'Modulation of the Bacterial Clearance Activity of Haemocytes from the Freshwater Mollusc, *Lymnaea Stagnalis*, by the Avian Schistosome, *Trichobilharzia Ocellata*', *Parasitology*, 109(3), pp. 299–310. Available at: https://doi.org/10.1017/S0031182000078331.

Nuno, V., João, G.M., Gabriel, R., J., B.P., Fátima, G. and M., C. da C.J. (2017) 'Praziquantel for Schistosomiasis: Single-Drug Metabolism Revisited, Mode of Action, and Resistance', *Antimicrobial Agents and Chemotherapy*, 61(5), pp. 10.1128/aac.02582-16. Available at: https://doi.org/10.1128/aac.02582-16.

Nyindo, M. and Lukambagire, A.H. (2015) 'Fascioliasis: An Ongoing Zoonotic Trematode Infection', *BioMed Research International*, 2015, p. 786195. Available at: https://doi.org/10.1155/2015/786195.

Ogunlade, S.T., Adekunle, A.I., Meehan, M.T. and McBryde, E.S. (2023) 'Quantifying the impact of *Wolbachia* releases on dengue infection in Townsville, Australia', *Scientific Reports*, 13(1), pp. 1–12. Available at: https://doi.org/10.1038/s41598-023-42336-2.

Osborne, S.E., Leong, Y.S., O'Neill, S.L. and Johnson, K.N. (2009) 'Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*', *PLoS Pathogens*, 5(11). Available at: https://doi.org/10.1371/journal.ppat.1000656.

Osório, J.B. *et al.* (2020) 'Mollusk microbiota shift during *Angiostrongylus cantonensis* infection in the freshwater snail *Biomphalaria glabrata* and the terrestrial slug *Phillocaulis soleiformis*', *Parasitology Research*, 119(8), pp. 2495–2503. Available at: https://doi.org/10.1007/s00436-020-06743-y.

Overstreet, R.M. and Lotz, J.M. (2016) 'Host–Symbiont Relationships: Understanding the Change from Guest to Pest', in *The Rasputin Effect: When Commensals and Symbionts Become Parasitic*. Springer, pp. 27–64. Available at: https://doi.org/10.1007/978-3-319-28170-4_2.

Paczesniak, D., Klappert, K., Kopp, K., Neiman, M., Seppälä, K., Lively, C.M. and Jokela, J. (2019) 'Parasite resistance predicts fitness better than fecundity in a natural population of the freshwater snail *Potamopyrgus antipodarum*', *Evolution*, 73(8), pp. 1634–1646. Available at: https://doi.org/10.1111/evo.13768.

Panova, M.A.Z., Varfolomeeva, M.A., Gafarova, E.R., Maltseva, A.L., Mikhailova, N.A. and Granovitch, A.I. (2023) 'First insights into the gut microbiomes and the diet of the *Littorina* snail ecotypes, a recently emerged marine evolutionary model', *Evolutionary Applications*, 16(2), pp. 365–378. Available at: https://doi.org/10.1111/eva.13447.

Parker, A. *et al.* (2022) 'Fecal microbiota transfer between young and aged mice reverses hallmarks of the aging gut, eye, and brain.', *Microbiome*, 10(1), p. 68. Available at: https://doi.org/10.1186/s40168-022-01243-w.

Pica-Mattoccia, L. and Cioli, D. (2004) 'Sex- and stage-related sensitivity of *Schistosoma mansoni* to in vivo and in vitro praziquantel treatment', *International Journal for Parasitology*, 34(4), pp. 527–

533. Available at: https://doi.org/10.1016/j.ijpara.2003.12.003.

Picquet, M., Ernould, J.C., Vercruysse, J., Southgate, V.R., Mbaye, A., Sambou, B., Niang, M. and Rollinson, D. (1996) 'The epidemiology of human schistosomiasis in the Senegal river basin.', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 90.

Pila, E.A., Gordy, M.A., Phillips, V.K., Kabore, A.L., Rudko, S.P. and Hanington, P.C. (2016) 'Endogenous growth factor stimulation of hemocyte proliferation induces resistance to *Schistosoma mansoni* challenge in the snail host', *Proceedings of the National Academy of Sciences*, 113(19), pp. 5305–5310. Available at: https://doi.org/10.1073/pnas.1521239113.

Pinto, S.B. *et al.* (2021) 'Effectiveness of *Wolbachia*-infected mosquito deployments in reducing the incidence of dengue and other *Aedes*-borne diseases in Niterói, Brazil: A quasi-experimental study.', *PLoS neglected tropical diseases*, 15(7), p. e0009556. Available at: https://doi.org/10.1371/journal.pntd.0009556.

Pointier, J.-P., Coustau, C., Rondelaud, D. and Theron, A. (2007) '*Pseudosuccinea columella* (Say 1817) (Gastropoda, Lymnaeidae), snail host of *Fasciola hepatica*: first record for France in the wild', *Parasitology Research*, 101(5), pp. 1389–1392. Available at: https://doi.org/10.1007/s00436-007-0656-y.

Polley, L. (2005) 'Navigating parasite webs and parasite flow: Emerging and re-emerging parasitic zoonoses of wildlife origin', *International Journal for Parasitology*, 35(11–12), pp. 1279–1294. Available at: https://doi.org/10.1016/j.ijpara.2005.07.003.

Portet, A. *et al.* (2019) 'Sympatric versus allopatric evolutionary contexts shape differential immune response in *Biomphalaria / Schistosoma* interaction', *PLOS Pathogens*. Edited by M. Knight, 15(3), p. e1007647. Available at: https://doi.org/10.1371/journal.ppat.1007647.

Portet, A., Toulza, E., Lokmer, A., Huot, C., Duval, D., Galinier, R. and Gourbal, B. (2021) 'Experimental infection of the *Biomphalaria glabrata* vector snail by *Schistosoma mansoni* parasites drives snail microbiota dysbiosis', *Microorganisms*, 9(5), p. 1084. Available at: https://doi.org/10.3390/microorganisms9051084.

Poulin, R., Kamiya, T. and Lagrue, C. (2019) 'Evolution, phylogenetic distribution and functional ecology of division of labour in trematodes', *Parasites and Vectors*, 12(1), pp. 1–10. Available at: https://doi.org/10.1186/s13071-018-3241-6.

Rajarajan, A., Wolinska, J., Walser, J.-C., Mäder, M. and Spaak, P. (2022) 'Infection by a eukaryotic gut parasite in wild *Daphnia* sp. associates with a distinct bacterial community', *FEMS Microbiology Ecology*, 98(10), p. fiac097. Available at: https://doi.org/10.1093/femsec/fiac097.

Rellstab, C., Karvonen, A., Louhi, K.R. and Jokela, J. (2013) 'Genotype-Specific vs. Cross-Reactive Host Immunity against a Macroparasite', *PLoS ONE*, 8(10), pp. 1–8. Available at: https://doi.org/10.1371/journal.pone.0078427.

Ridaura, V.K. *et al.* (2013) 'Gut Microbiota from Twins Discordant for Obesity Modulate Metabolism in Mice', *Science*, 341(6150), p. 1241214. Available at: https://doi.org/10.1126/science.1241214.

Ripple, W.J. *et al.* (2023) 'Collapse of the world's largest herbivores', *Science Advances*, 1(4), p. e1400103. Available at: https://doi.org/10.1126/sciadv.1400103.

Rodman, J.E. and Cody, J.H. (2003) 'The Taxonomic Impediment Overcome: NSF's Partnerships for Enhancing Expertise in Taxonomy (PEET) as a Model', *Systematic Biology*, 52(3), pp. 428–435. Available at: http://www.jstor.org/stable/3651119.

Rohr, J.R. *et al.* (2008) 'Agrochemicals increase trematode infections in a declining amphibian species', *Nature*, 455, pp. 1235–1239. Available at: https://doi.org/10.1038/nature07281.

Rohr, J.R. *et al.* (2023) 'A planetary health innovation for disease, food and water challenges in Africa.', *Nature*, 619(7971), pp. 782–787. Available at: https://doi.org/10.1038/s41586-023-06313-z.

Rohwer, F., Seguritan, V., Azam, F. and Knowlton, N. (2002) 'Diversity and distribution of coral-associated bacteria', *Marine Ecology Progress Series*, 243, pp. 1–10.

Rondelaud, D., Vignoles, P. and Dreyfuss, G. (2007) 'Parasite development and visceral pathology in *Galba truncatula* co-infected with *Fasciola hepatica* and *Paramphistomum daubneyi*', *Journal of Helminthology*, 81(3), pp. 317–322. Available at: https://doi.org/10.1017/S0022149X07818542.

Rondelaud, D., Vignoles, P. and Dreyfuss, G. (2022) 'Changes in the Populations of Two Lymnaeidae and Their Infection by *Fasciola hepatica* and/or *Calicophoron daubneyi* over the Past 30 Years in Central France', *Animals*, 12(24). Available at: https://doi.org/10.3390/ani12243566.

Rooks, M.G. and Garrett, W.S. (2016) 'Gut microbiota, metabolites and host immunity', *Nature Reviews Immunology*, 16(6), pp. 341–352. Available at: https://doi.org/10.1038/nri.2016.42.

Salafsky, B., Ramaswamy, K., He, Y.X., Li, J. and Shibuya, T. (1999) 'Development and evaluation of lipodeet, a new long-acting formulation of N, N-diethyl-m-toluamide (DEET) for the revention of schistosomiasis', *American Journal of Tropical Medicine and Hygiene*, 61(5), pp. 743–750. Available at: https://doi.org/10.4269/ajtmh.1999.61.743.

Saleem, M., Fetzer, I., Harms, H. and Chatzinotas, A. (2013) 'Diversity of protists and bacteria determines predation performance and stability', *ISME Journal*, 7(10), pp. 1912–1921. Available at: https://doi.org/10.1038/ismej.2013.95.

Salloum, P.M., Jorge, F., Dheilly, N.M. and Poulin, R. (2023a) 'Adoption of alternative life cycles in a parasitic trematode is linked to microbiome differences', *Biology Letters*, 19(6), p. 20230091. Available at: https://doi.org/10.1098/rsbl.2023.0091.

Salloum, P.M., Jorge, F. and Poulin, R. (2023b) 'Different trematode parasites in the same snail host: Species-specific or shared microbiota?', *Molecular ecology*, 32(19), pp. 5414–5428. Available at: https://doi.org/10.1111/mec.17111.

Sato, T., Watanabe, K., Kanaiwa, M., Niizuma, Y., Harada, Y. and Lafferty, K.D. (2011) 'Nematomorph parasites drive energy flow through a riparian ecosystem', *Ecology*, 92(1), pp. 201–207. Available at: https://doi.org/https://doi.org/10.1890/09-1565.1.

Schellenberg, J. *et al.* (2020) 'The Bacterial Microbiome of the Long-Term Aquarium Cultured High-Microbial Abundance Sponge *Haliclona cnidata* – Sustained Bioactivity Despite Community Shifts Under Detrimental Conditions', *Frontiers in Marine Science*, 7. Available at: https://doi.org/10.3389/fmars.2020.00266.

Schniebs, K., Glöer, P., Vinarski, M. and Hundsdoerfer, A. (2016) 'A barcode pitfall in Palaearctic *Stagnicola* specimens (Mollusca: Lymnaeidae): Incongruence of mitochondrial genes, a nuclear marker and morphology', *North-Western Journal of Zoology*, 12, pp. 239–254.

Schols, R., Carolus, H., Hammoud, C., Mulero, S., Mudavanhu, A. and Huyse, T. (2019) 'A rapid diagnostic multiplex PCR approach for xenomonitoring of human and animal schistosomiasis in a "One Health" context', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 113(11), pp. 722–729. Available at: https://doi.org/10.1093/trstmh/trz067.

Schols, R., Mudavanhu, A., Carolus, H., Hammoud, C., Muzarabani, K.C., Barson, M. and Huyse, T. (2020) 'Exposing the Barcoding Void: An Integrative Approach to Study Snail-Borne Parasites in a One Health Context', *Frontiers in Veterinary Science*, 7, p. 1046. Available at: https://doi.org/10.3389/fvets.2020.605280.

Schols, R., Carolus, H., Hammoud, C., Muzarabani, K.C., Barson, M. and Huyse, T. (2021) 'Invasive snails, parasite spillback, and potential parasite spillover drive parasitic diseases of *Hippopotamus amphibius* in artificial lakes of Zimbabwe', *BMC Biology*, 19(1), p. 160. Available at: https://doi.org/10.1186/s12915-021-01093-2.

Schols, R., Vanoverberghe, I., Huyse, T. and Decaestecker, E. (2023) 'Host-bacteriome transplants of the schistosome snail host *Biomphalaria glabrata* reflect species-specific associations', *FEMS Microbiology Ecology*, 99(9), p. fiad101. Available at: https://doi.org/10.1093/femsec/fiad101.

Schols, R., Smitz, N., Vanderheyden, A. and Huyse, T. (2024) 'Expanding the swimmer's itch pool of the Benelux: a first record of the neurotropic *Trichobilharzia regenti* and potential link to human infection', *Parasites and Vectors*, 17(1), pp. 1–11. Available at: https://doi.org/10.1186/s13071-024-06218-4.

Schols, R., Henrard, A., Brecko, J., Mudavanhu, A., Goossens, E., Steffanie, N., Clegg, S., Vanhove, M.P.M. and Huyse, T. (2025a) 'Innovating stomach fluke identification: an integrative approach combining Micro-CT imaging and molecular tools', *International Journal for Parasitology*, in press. Available at: https://doi.org/10.1016/j.ijpara.2025.05.002.

Schols, R., Hammoud, C., Bisschop, K., Vanoverberghe, I., Huyse, T. and Decaestecker, E. (2025b) 'Schistosome species, parasite development, and co-infection combinations determine microbiome dynamics in the snail Biomphalaria glabrata', *Research Square - preprint* [Preprint]. Available at: https://doi.org/10.21203/rs.3.rs-6646903/v1.

Schols, R. and Huyse, T. (2024) '*Fasciola nyanzae*', *Trends in Parasitology*, 40(6), pp. 527–528. Available at: https://doi.org/10.1016/j.pt.2024.01.007.

Schweizer, G., Braun, U., Deplazes, P. and Torgerson, P.R. (2005) 'Estimating the financial losses due to bovine fasciolosis in Switzerland', *Veterinary Record*, 157(7), pp. 188–193. Available at: https://doi.org/10.1136/vr.157.7.188.

Sender, R., Fuchs, S. and Milo, R. (2016) 'Revised Estimates for the Number of Human and Bacteria Cells in the Body', *PLoS Biology*, 14(8), pp. 1–14. Available at: https://doi.org/10.1371/journal.pbio.1002533.

Sène, M., Southgate, V.R. and Vercruysse, J. (2004) '*Bulinus truncatus*, intermediate host of Schistosoma haematobium in the Senegal River Basin (SRB).', *Bulletin de la Societe de pathologie exotique*, 97(1), pp. 29–32.

Sengupta, M.E. *et al.* (2019) 'Environmental DNA for improved detection and environmental surveillance of schistosomiasis', *Proceedings of the National Academy of Sciences*, 116(18), pp. 8931–8940. Available at: https://doi.org/10.1073/pnas.1815046116.

Sergaki, C., Lagunas, B., Lidbury, I., Gifford, M.L. and Schäfer, P. (2018) 'Challenges and Approaches in Microbiome Research: From Fundamental to Applied', *Frontiers in Plant Science*, 9, p. 1205.

Sey, O. (1991) *CRC Handbook of the Zoology of Amphistomes*. CRC Press. Available at: https://doi.org/10.1201/9781315150871.

Shi, H., Yu, X. and Cheng, G. (2023) 'Impact of the microbiome on mosquito-borne diseases', *Protein*

& cell, 14(10), pp. 743–761. Available at: https://doi.org/10.1093/procel/pwad021.

Singh, P., Ballmer, D.N., Laubscher, M. and Schärer, L. (2020) 'Successful mating and hybridisation in two closely related flatworm species despite significant differences in reproductive morphology and behaviour', *Scientific Reports*, 10(1), pp. 1–16. Available at: https://doi.org/10.1038/s41598-020-69767-5.

Smith, K.F., Acevedo-Whitehouse, K. and Pedersen, A.B. (2009) 'The role of infectious diseases in biological conservation', *Animal Conservation*, 12(1), pp. 1–12. Available at: https://doi.org/10.1111/j.1469-1795.2008.00228.x.

Snyder, J.E., Smart, J.C., Goeb, J.C. and Tschirley, D.L. (2018) 'Pesticide use in Sub-Saharan Africa: Estimates, Projections, and Implications in the Context of Food System Transformation', in *Agricultural and Applied Economics Association (AAEA)*. Washington, D.C. Available at: https://doi.org/10.22004/ag.econ.273900.

Sokolow, S.H. *et al.* (2016) 'Global Assessment of Schistosomiasis Control Over the Past Century Shows Targeting the Snail Intermediate Host Works Best', *PLoS Neglected Tropical Diseases*, 10(7), pp. 1–19. Available at: https://doi.org/10.1371/journal.pntd.0004794.

Sokolow, S.H. *et al.* (2017) 'Nearly 400 million people are at higher risk of schistosomiasis because dams block the migration of snail-eating river prawns', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1722), p. 20160127. Available at: https://doi.org/10.1098/rstb.2016.0127.

Sokolow, S.H., Wood, C.L., Jones, I.J., Lafferty, K.D., Kuris, A.M., Hsieh, M.H. and De Leo, G.A. (2018) 'To Reduce the Global Burden of Human Schistosomiasis, Use "Old Fashioned" Snail Control', *Trends in Parasitology*, 34(1), pp. 23–40. Available at: https://doi.org/10.1016/j.pt.2017.10.002.

Soldánová, M., Selbach, C., Kalbe, M., Kostadinova, A. and Sures, B. (2013) 'Swimmer's itch: etiology, impact, and risk factors in Europe.', *Trends in parasitology*, 29(2), pp. 65–74. Available at: https://doi.org/10.1016/j.pt.2012.12.002.

Soldánová, M., Selbach, C. and Sures, B. (2016) 'The Early Worm Catches the Bird? Productivity and Patterns of *Trichobilharzia szidati* Cercarial Emission from *Lymnaea stagnalis*', *PLOS ONE*, 11(2), p. e0149678.

Song, X., Wang, M., Dong, L., Zhu, H. and Wang, J. (2018) 'PGRP-LD mediates *A. stephensi* vector competency by regulating homeostasis of microbiota-induced peritrophic matrix synthesis', *PLoS Pathogens*, 14(2), pp. 1–18. Available at: https://doi.org/10.1371/journal.ppat.1006899.

Southgate, V.R., de Clercq, D., Sène, M., Rollinson, D., Ly, A. and Vercruysse, J. (2000) 'Observations on the compatibility between *Bulinus*spp. and *Schistosoma haematobium* in the Senegal River basin.', *Annals of tropical medicine and parasitology*, 94(2), pp. 157–164. Available at: https://doi.org/10.1080/00034980057491.

Stacy, A. *et al.* (2021) 'Infection trains the host for microbiota-enhanced resistance to pathogens', *Cell*, 184(3), pp. 615-627.e17. Available at: https://doi.org/10.1016/j.cell.2020.12.011.

Stears, K., McCauley, D.J., Finlay, J.C., Mpemba, J., Warrington, I.T., Mutayoba, B.M., Power, M.E., Dawson, T.E. and Brashares, J.S. (2018) 'Effects of the hippopotamus on the chemistry and ecology of a changing watershed', *Proceedings of the National Academy of Sciences of the United States of America*, 115(22), pp. E5028–E5037. Available at: https://doi.org/10.1073/pnas.1800407115.

Stock, W. *et al.* (2021) 'Human impact on symbioses between aquatic organisms and microbes',

Aquatic Microbial Ecology, 87, pp. 113–138. Available at: https://doi.org/10.3354/ame01973.

Stringer, A.P. and Linklater, W. (2014) 'Everything in moderation: Principles of Parasite control for wildlife conservation', *BioScience*, 64(10), pp. 932–937. Available at: https://doi.org/10.1093/biosci/biu135.

Strong, E.E., Gargominy, O., Ponder, W.F. and Bouchet, P. (2008) 'Global diversity of gastropods (Gastropoda; Mollusca) in freshwater', *Hydrobiologia*, 595, pp. 149–166. Available at: https://doi.org/10.1007/s10750-007-9012-6.

Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., Kubo, C. and Koga, Y. (2004) 'Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice.', *The Journal of physiology*, 558(Pt 1), pp. 263–275. Available at: https://doi.org/10.1113/jphysiol.2004.063388.

Sun, X., Hong, J., Ding, T., Wu, Z. and Lin, D. (2024) 'Snail microbiota and snail–schistosome interactions: axenic and gnotobiotic technologies', *Trends in Parasitology*, 40(3), pp. 241–256. Available at: https://doi.org/10.1016/j.pt.2024.01.002.

Takacs-Vesbach, C., King, K., Van Horn, D., Larkin, K. and Neiman, M. (2016) 'Distinct Bacterial Microbiomes in Sexual and Asexual *Potamopyrgus antipodarum*, a New Zealand Freshwater Snail', *PLOS ONE*, 11(8), p. e0161050. Available at: https://doi.org/10.1371/journal.pone.0161050.

Talla, I., Kongs, A., Verle, P., Belot, J., Sarr, S. and Coll, A.M. (1990) 'Outbreak of intestinal schistosomiasis in the Senegal River Basin.', *Annales de la Société Belge de Médecine Tropicale*, 70(3), pp. 173–180.

van Tatenhove-Pel, R.J. *et al.* (2021) 'Microbial competition reduces metabolic interaction distances to the low µm-range', *ISME Journal*, 15(3), pp. 688–701. Available at: https://doi.org/10.1038/s41396-020-00806-9.

Tavalire, H.F., Blouin, M.S. and Steinauer, M.L. (2016) 'Genotypic variation in host response to infection affects parasite reproductive rate', *International Journal for Parasitology*, 46(2), pp. 123–131. Available at: https://doi.org/https://doi.org/10.1016/j.ijpara.2015.10.001.

Taylor, M.J., Bandi, C. and Hoerauf, A. (2005) 'Wolbachia Bacterial Endosymbionts of Filarial Nematodes', in J.R. Baker, R. Muller, and D.B.T.-A. in P. Rollinson (eds) *Advances in Parasitology*. Elsevier, pp. 245–284. Available at: https://doi.org/10.1016/S0065-308X(05)60004-8.

Taylor, M.J., Hoerauf, A. and Bockarie, M. (2010) 'Lymphatic filariasis and onchocerciasis', *The Lancet*, 376(9747), pp. 1175–1185. Available at: https://doi.org/10.1016/S0140-6736(10)60586-7.

Theis, K.R. *et al.* (2016) 'Getting the Hologenome Concept Right: an Eco-Evolutionary Framework for Hosts and Their Microbiomes', *mSystems*, 1(2), pp. 1–6. Available at: https://doi.org/10.1128/msystems.00028-16.

Theron, A., Rognon, A., Gourbal, B. and Mitta, G. (2014) 'Multi-parasite host susceptibility and multi-host parasite infectivity: A new approach of the Biomphalaria glabrata/Schistosoma mansoni compatibility polymorphism', *Infection, Genetics and Evolution*, 26, pp. 80–88. Available at: https://doi.org/10.1016/j.meegid.2014.04.025.

Théron, A. (1989) 'Hybrids between Schistosoma mansoni and S. rodhaini: characterization by cercarial emergence rhythms', *Parasitology*, 99, pp. 225–228.

Thompson, R.C.A., Lymbery, A.J. and Smith, A. (2010) 'Parasites, emerging disease and wildlife

conservation', *International Journal for Parasitology*, 40(10), pp. 1163–1170. Available at: https://doi.org/10.1016/j.ijpara.2010.04.009.

Toledo, R. and Fried, B. (2011) Biomphalaria *snails and larval trematodes*. 1st edn. Edited by T. Rafael and F. Bernard. Springer New York, NY. Available at: https://doi.org/10.1007/978-1-4419-7028-2.

Toledo, R. and Fried, B. (2014) *Digenetic Trematodes*. Edited by R. Toledo and B. Fried. New York, NY: Springer New York (Advances in Experimental Medicine and Biology). Available at: https://doi.org/10.1007/978-1-4939-0915-5.

Tompkins, D.M., Dunn, A.M., Smith, M.J. and Telfer, S. (2011) 'Wildlife diseases: From individuals to ecosystems', *Journal of Animal Ecology*, 80(1), pp. 19–38. Available at: https://doi.org/10.1111/j.1365-2656.2010.01742.x.

Tortajada, C., Altinbilek, D. and Biswas, A.K. (2012) *Impacts of Large Dams: A Global Assessment*. 1st edn. Edited by C. Tortajada, D. Altinbilek, and A.K. Biswas. Springer Berlin, Heidelberg. Available at: https://doi.org/10.1007/978-3-642-23571-9.

Tumusiime, J., Valderrama Bhraunxs, N., Kagoro-Rugunda, G., Namirembe, D., Albrecht, C., Twongyirwe, R., Tolo, C.U., Jacobs, L. and Huyse, T. (2024) 'Citizens can help to map putative transmission sites for snail-borne diseases', *PLoS Neglected Tropical Diseases*, 18(4), pp. 1–25. Available at: https://doi.org/10.1371/journal.pntd.0012062.

Vázquez, A.A., Sánchez, J., Pointier, J.P., Théron, A. and Hurtrez-Boussès, S. (2014) '*Fasciola hepatica* in Cuba: Compatibility of different isolates with two intermediate snail hosts, *Galba cubensis* and *Pseudosuccinea columella*', *Journal of Helminthology*, 88(4), pp. 434–440. Available at: https://doi.org/10.1017/S0022149X13000382.

Vázquez, A.A. *et al.* (2019) 'Reviewing Fasciola hepatica transmission in the West Indies and novel perceptions from experimental infections of sympatric vs. allopatric snail/fluke combinations', *Veterinary Parasitology*, 275, p. 108955. Available at: https://doi.org/https://doi.org/10.1016/j.vetpar.2019.108955.

Verhulst, N.O. *et al.* (2011) 'Composition of human skin microbiota affects attractiveness to malaria mosquitoes', *PLoS ONE*, 6(12). Available at: https://doi.org/10.1371/journal.pone.0028991.

Vinarski, M. V (2020) 'Roots of the taxonomic impediment: Is the "integrativeness" a remedy?', *Integrative Zoology*, 15(1), pp. 2–15. Available at: https://doi.org/10.1111/1749-4877.12393.

Voulgari-Kokota, A., Beukeboom, L.W., Wertheim, B. and Salles, J.F. (2022) 'Houseflies harbor less diverse microbiota under laboratory conditions but maintain a consistent set of host-associated bacteria', *Scientific Reports*, 12(1), p. 11132. Available at: https://doi.org/10.1038/s41598-022-15186-7.

Wang, S., Ghosh, A.K., Bongio, N., Stebbings, K.A., Lampe, D.J. and Jacobs-Lorena, M. (2012) 'Fighting malaria with engineered symbiotic bacteria from vector mosquitoes', *Proceedings of the National Academy of Sciences of the United States of America*, 109(31), pp. 12734–12739. Available at: https://doi.org/10.1073/pnas.1204158109.

Wang, S., Dos-santos, A.L.A., Huang, W., Liu, K.C., Oshaghi, M.A., Wei, G., Agre, P. and Jacobs-lorena, M. (2017) 'Driving mosquito refractoriness to *Plasmodium falciparum* with Engineered Symbiotic Bacteria', *Science*, 357, pp. 1399–1402.

Webster, B.L., Diaw, O.T., Seye, M.M., Webster, J.P. and Rollinson, D. (2013) 'Introgressive

Hybridization of *Schistosoma haematobium* Group Species in Senegal: Species Barrier Break Down between Ruminant and Human Schistosomes', *PLOS Neglected Tropical Diseases*, 7(4), p. e2110. Available at: https://doi.org/10.1371/journal.pntd.0002110.

Webster, J.P., Gower, C.M., Knowles, S.C.L., Molyneux, D.H. and Fenton, A. (2016) 'One health - an ecological and evolutionary framework for tackling Neglected Zoonotic Diseases', *Evolutionary Applications*, 9(2), pp. 313–333. Available at: https://doi.org/10.1111/eva.12341.

Webster, J.P., Hoffman, J.I. and Berdoy, M. (2003) 'Parasite infection, host resistance and mate choice: Battle of the genders in a simultaneous hermaphrodite', *Proceedings of the Royal Society B: Biological Sciences*, 270(1523), pp. 1481–1485. Available at: https://doi.org/10.1098/rspb.2003.2354.

Webster, J.P. and Woolhouse, M.E.J. (1998) 'Selection And Strain Specificity Of Compatibility Between Snail Intermediate Hosts And Their Parasitic Schistosomes', *Evolution*, 52(6), pp. 1627–1634. Available at: https://doi.org/10.1111/j.1558-5646.1998.tb02243.x.

WHO (1965) 'Molluscicide screening and evaluation.', *Bulletin of the World Health Organization*, 33(4), pp. 567–581.

WHO (2016) 'Schistosomiasis and soil-transmitted helminthiases: number of people treated in 2015.', *Weekly epidemiological record*, 91(49/50), pp. 585–595.

WHO (2020) *Ending the neglect to attain the sustainable development goals: a road map for neglected tropical diseases 2021–2030.*, *Who*.

WHO (2023) *Schistosomiasis [fact sheet]*. Available at: https://www.who.int/news-room/fact-sheets/detail/schistosomiasis#:~:text=Schistosomiasis is an acute and chronic parasitic disease caused by,will reduce and prevent morbidity.

Will, K.W., Mishler, B.D. and Wheeler, Q.D. (2005) 'The Perils of DNA Barcoding and the Need for Integrative Taxonomy', *Systematic Biology*, 54(5), pp. 844–851. Available at: https://doi.org/10.1080/10635150500354878.

Wolinska, J. and King, K.C. (2009) 'Environment can alter selection in host-parasite interactions', *Trends in Parasitology*, 25(5), pp. 236–244. Available at: https://doi.org/10.1016/j.pt.2009.02.004.

Wong, P.K., Kwong, K.L. and Qiu, J.-W. (2009) 'Complex interactions among fish, snails and macrophytes: implications for biological control of an invasive snail', *Biological Invasions*, 11(10), pp. 2223–2232. Available at: https://doi.org/10.1007/s10530-008-9378-z.

Woo, V. and Alenghat, T. (2022) 'Epigenetic regulation by gut microbiota.', *Gut microbes*, 14(1), p. 2022407. Available at: https://doi.org/10.1080/19490976.2021.2022407.

Wood, C.L. *et al.* (2023) 'A reconstruction of parasite burden reveals one century of climate-associated parasite decline', *Proceedings of the National Academy of Sciences*, 120(3), p. e2211903120. Available at: https://doi.org/10.1073/pnas.2211903120.

Wood, C.L. and Vanhove, M.P.M. (2023) 'Is the world wormier than it used to be? We'll never know without natural history collections', *Journal of Animal Ecology*, 92(2), pp. 250–262. Available at: https://doi.org/https://doi.org/10.1111/1365-2656.13794.

Wright, C.A. and Ross, G.C. (1980) 'Hybrids between Schistosoma haematobium and S. mattheei and their identification by isoelectric focusing of enzymes', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 74(3), pp. 326–332.

Wright, W.H. (1972) 'A consideration of economic impact of schistosomiasis.', *Bulletin of the World Health Organization*, 47(5), pp. 559–565.

Wuerthner, V.P., Hua, J. and Hoverman, J.T. (2017) 'The benefits of coinfection: trematodes alter disease outcomes associated with virus infection', *Journal of Animal Ecology*, 86(4), pp. 921–931. Available at: https://doi.org/10.1111/1365-2656.12665.

Yellowlees, D., Rees, T.A. V. and Leggat, W. (2008) 'Metabolic interactions between algal symbionts and invertebrate hosts', *Plant, Cell and Environment*, 31(5), pp. 679–694. Available at: https://doi.org/10.1111/j.1365-3040.2008.01802.x.

You, H., Cai, P., Tebeje, B., Li, Y. and McManus, D. (2018) 'Schistosome Vaccines for Domestic Animals', *Tropical Medicine and Infectious Disease*, 3(2), p. 68. Available at: https://doi.org/10.3390/tropicalmed3020068.

Zaneveld, J.R., McMinds, R. and Vega Thurber, R. (2017) 'Stress and stability: applying the Anna Karenina principle to animal microbiomes', *Nature Microbiology*, 2(9), p. 17121. Available at: https://doi.org/10.1038/nmicrobiol.2017.121.

Zélé, F., Nicot, A., Berthomieu, A., Weill, M., Duron, O. and Rivero, A. (2014) '*Wolbachia* increases susceptibility to *Plasmodium* infection in a natural system', *Proceedings of the Royal Society B: Biological Sciences*, 281(1779), p. 20132837. Available at: https://doi.org/10.1098/rspb.2013.2837.

Zhang, J., Pitol, A.K., Braun, L., Hazell, L. and Templeton, M.R. (2022) 'The efficacy of soap against schistosome cercariae: A systematic review', *PLoS Neglected Tropical Diseases*, 16(10), pp. 1–17. Available at: https://doi.org/10.1371/journal.pntd.0010820.

Zhou, S., Gravekamp, C., Bermudes, D. and Liu, K. (2018) 'Tumour-targeting bacteria engineered to fight cancer', *Nature Reviews Cancer*, 18(12), pp. 727–743. Available at: https://doi.org/10.1038/s41568-018-0070-z.

Ziegler, A., Ogurreck, M., Steinke, T., Beckmann, F., Prohaska, S. and Ziegler, A. (2010) 'Opportunities and challenges for digital morphology', *Biology Direct*, 5(1), p. 45. Available at: https://doi.org/10.1186/1745-6150-5-45.

Zielinski, F.U., Pernthaler, A., Duperron, S., Raggi, L., Giere, O., Borowski, C. and Dubilier, N. (2009) 'Widespread occurrence of an intranuclear bacterial parasite in vent and seep bathymodiolin mussels', *Environmental Microbiology*, 11(5), pp. 1150–1167. Available at: https://doi.org/10.1111/j.1462-2920.2008.01847.x.

Zilber-Rosenberg, I. and Rosenberg, E. (2008) 'Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution', *FEMS Microbiology Reviews*, 32(5), pp. 723–735. Available at: https://doi.org/10.1111/j.1574-6976.2008.00123.x.

Zimmermann, D., Randolf, S., Metscher, B.D. and Aspöck, U. (2011) 'The function and phylogenetic implications of the tentorium in adult Neuroptera (Insecta)', *Arthropod Structure & Development*, 40(6), pp. 571–582. Available at: https://doi.org/https://doi.org/10.1016/j.asd.2011.06.003.

Zimmermann, I.R., Alves Fernandes, R.R., Santos da Costa, M.G., Pinto, M. and Peixoto, H.M. (2024) 'Simulation-based economic evaluation of the *Wolbachia* method in Brazil: a cost-effective strategy for dengue control', *The Lancet Regional Health – Americas*, 35, p. 100783. Available at: https://doi.org/10.1016/j.lana.2024.100783.