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Preprint · March 2024

DOI: 10.21203/rs.3.rs-4145796/v1

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Research Article

Keywords: Biodiversity, Parasites, Hosts Abundance, Trematodes, Schistosomiasis, Antagonistic Interaction, Transmission

Posted Date: April 4th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4145796/v1

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Declarations

This study was a part of a large project investigating the *Schistosoma* invasive capacity (Grant number: EDCTP-TMA2018CDF-2370-Sen_Hybrid_Invasion).

Ethics approval and consent to participate

The project has received approval from the National Ethical Committee (CNERS) of Senegal (agreement number: 00061/MSAS/CNERS/SP).

Consent for publication

"Not applicable"

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

This project obtained from the Nagoya office in Senegal, an exemption from authorization of access and use of genetic resources (number: 001339 of November 15, 2021, reference: V/L du 28 octobre 2021) by the Competent National Authority (Directorate of National Parks of Senegal).

Competing interests

The authors declare that they have no competing interests

Funding:

This study was supported in part by the European and Developing Countries Clinical Trials Partnership (EDCTP2) programme (TMA2018CDF-2370), supported by the European Union. It was funded by the French Agency for Food, Environmental and Occupational Health & Safety (PNRES 2019/1/059 Molrisk) and the Occitanie Region (Schistodiag program). It was also supported by the European and Developing Countries Clinical Trials Partnership (EDCTP2) programme (TMA2018CDF-2370), supported by the European Union. This study was carried out with the support of LabEx CeMEB, an ANR "Investissements d'avenir" program (ANR-10- LABX-04-01), and within the framework of the "Laboratoire d'Excellence (LABEX)" TULIP (ANR-10LABX-41).

Authors' contributions

PD have made substantial contributions to the conception, design of the work, acquisition, analysis, and interpretation of data. He was a major contributor in writing the manuscript, have approved the submitted version, and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the

work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

BH have made substantial contributions to the creation of the mathematical model used in the work, the analysis, and interpretation of data. He was a major contributor in writing the manuscript, have approved the submitted version, and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

JFA have made substantial contributions to the acquisition of the molecular data. He have approved the submitted version, and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

JB have made substantial contributions to the conception, design of the work, acquisition, and interpretation of data. He have approved the submitted version, and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

BS have made substantial contributions to the acquisition of the field data. He have approved the submitted version, and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

OR have made substantial contributions to the conception, design of the work, acquisition, and interpretation of data. He was a major contributor in writing the manuscript, have approved the submitted version, and have agreed both to be personally accountable for the author's own

contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

Acknowledgments

The authors warmly thank the Bio-Environnement platform (University of Perpignan Via Domitia) for sequencing and bioinformatics services. We would like to thank Pr Doudou Sow who allowed us to process the samples and snail experimental infestations in the laboratory of Parasitology and Mycology of the Gaston Berger University and EDCTP2 Program supported by the European Union, which supported our flight and field works in Senegal. Furthermore, we thank Alassane Dit Baye Ndiaye and Modou Diop for their help in the field.

Keywords (3 to 10)

Biodiversity, Parasites, Hosts Abundance, Trematodes, Schistosomiasis, Antagonistic Interaction, Transmission

1. Abstract (350 mots MAX)

Background:

Combating infectious diseases and halting biodiversity loss are intertwined challenges crucial to ensure global health. Biodiversity can constrain the spread of vector-borne pathogens circulation, necessitating a deeper understanding of ecological mechanisms underlying this pattern. Our study evaluates the relative importance of biodiversity and the abundance of *Bulinus truncatus*, a major intermediate host for the trematode *Schistosoma haematobium* on the circulation of this human pathogen at aquatic transmission sites.

Methods: We combined mathematical modelling and a molecular based empirical study to specifically assess the effect of co-infections between *S. haematobium* and other trematodes within their *B.*

truncatus snail hosts; and *B. truncatus* abundance at transmission sites, on the production of *S. haematobium* infective cercariae stages released into the aquatic environment.

Results:

Our modelling approach shows that more competitive trematode species exploiting *B. truncatus* as an intermediate host at the transmission site level leads to higher co-infection rates within snail hosts, subsequently reducing the production of *S. haematobium* cercariae. Conversely, an increase in *B. truncatus* abundance results in lower co-infection rates, and a higher proportion of *S. haematobium* cercariae released into the environment. Our empirical data from the field support these findings, indicating a significant negative effect of local trematode species richness and co-infection rates on the dominance of S. *haematobium*, while *B. truncatus* abundance positively influences *S. haematobium* dominance.

Conclusions:

Our study highlights the importance of biodiversity in influencing the transmission of *S. haematobium* through the effect of antagonistic interactions between trematodes within bulinid snail hosts. This effect intensifies when *B. truncatus* populations are low, promoting co-infections within snails. In line with the need for concrete applications of the One Health concept, we advocate for maintaining high global freshwater biodiversity to sustain global trematode diversity at transmission sites, offering a cost-effective means to significantly reduce schistosome prevalence and intensity while preserving aquatic ecosystem services.

2. Introduction

One of the major consequences of global change is a drastic modification of the circulation of pathogens and an increase in disease emergences and outbreaks worldwide (1–3). The causes of these global-scale increases are multifactorial and the profound changes in biodiversity observed globally contribute significantly (4). On one hand, while some populations/species are extending, including

humans and all domesticated species upon which human societies rely, most populations of most species are declining (5,6). On the other hand, it is generally assumed that parasite transmission increases with host density although generally not linearly (7). As a result, the growth and densification of human populations and domestic organisms contribute to the emergence and circulation of numerous pathogens sometimes causing severe health and economic issues which tend to extend at a planetary scale due to global international trades (8). Moreover, the reduction in biodiversity in an existing community of hosts and parasites can lead to a decrease in the abundance of non-competent hosts, which, when present, mitigate the circulation of parasites through a dilution effect (9). The generality and ecological importance of this dilution effect is however widely debated (10–12). Additionally, host diversity in a community can support the co-existence of several circulating pathogen species, between which antagonistic (or beneficial) interactions within their hosts or vectors can significantly hamper (or facilitate) the circulation of least competitive pathogens (13).

Both facilitating and antagonistic interactions exist between trematodes. These parasitic worms display complex life cycles more generally including a vertebrate organism as definitive hosts and several successive invertebrate and/or vertebrate organisms are intermediate hosts including most of the time (aquatic) snails as first intermediate hosts. Such complex life cycles provide opportunities for interactions between trematode species and modulation of their transmission dynamics when coinfection occur especially in snail species that generally serve as amplifiers of trematodes in the environment. Some trematodes are causative agents of severe forms of Human and livestock diseases such as *Schistosoma* species. Interactions between *Schistosoma* species and other trematodes were historically documented with a focus on antagonistic interactions that could hamper their transmission as a mean of potential biological control strategies (14).

In fact, accruing evidence suggest that *Schistosoma* species that develop into sporocysts within their snail hosts are considered as moderate to bad competitors compared to other trematode species (13). Moreover, other trematode species that develop into rediae within snail hosts can predate the co-occuring *Schistosoma* sporocysts under development while consuming snail tissues (15). At natural

153 transmission sites of *Schistosoma* species, the importance of such antagonist interactions on the
154 circulation of *Schistosoma* species is generally underappreciated. Importantly however the snail
155 intermediate hosts used by some *Schistosoma* species (e.g. *Biomphalaria spp.*, *Bulinus spp.*) generally
156 host numerous trematode species hence providing opportunities for many intra-host interactions that
157 involve *Schistosoma* species. For example, up to 29 trematode species have been identified in natural
158 populations of *Biomphalaria pfeifferi* and *Biomphalaria sudanica* established over a constrained
159 geographical scale, these two snail species being important hosts for *Schistosoma mansoni* in eastern
160 Africa (13). Moreover, and as previously mentioned, *Schistosoma* species harbor low competitiveness
161 compared to many other species of trematodes (13). In line with these two characteristics, recent
162 modelling approaches predict over 50% decrease in the transmission of *S. mansoni* in the presence of
163 some highly competitive trematodes coinfecting *B. pfeifferi* – such as *Calicophoron sukari*, a widely
164 distributed parasite across livestock (13).

165 As a simple baseline, the prevalence of coinfection in a host population harbouring two
166 parasites equals the product of the prevalence of single infection of each parasite in the population
167 (15). However, coinfection rates observed *in natura* are generally lower than this theoretical
168 expectation. Interspecific competition leading to the rapid exclusion of one interacting species,
169 recruitment heterogeneity either due to random or parasite behaviours that limit co-infections,
170 immune priming mechanisms and high cost of co-infection on hosts fitness, may explain the departure
171 of this theoretical expectation as generally observed *in natura* (15,16). Intriguingly, the effect of host
172 abundance on co-infection rates is generally neglected since this theoretical expectation relies on the
173 relative prevalence of co-infection, which corresponds to the proportion of hosts that are infected (or
174 co-infected) among all hosts present in the environment. However, according to the generally
175 acknowledged density-dependent nature of pathogen transmission, it is expected that host density
176 also influences the co-infection rates, the occurrence of antagonistic interactions and ultimately the
177 transmission of less competitive parasites. If so, this could constitute an additional indirect mechanism

by which host density is positively correlated with parasite transmission by reducing co-infections and a release from antagonistic interactions.

Using the *Schistosoma haematobium* – *Bulinus truncatus* system as a model, the objective of this study is to assess the relative importance of the density of *B. truncatus* at transmission sites and the diversity of trematodes that use *B. truncatus* on the risk of transmission of *S. haematobium*. *Schistosoma haematobium* is a trematode species causing the urogenital form of bilharziasis in Humans mainly in the tropical regions in Africa (17). It displays a complex two-host lifecycle that involves humans as vertebrate hosts, in which the adults develop and reproduce sexually resulting in the production of thousands of eggs that are released in the environment through the urinary system of the host; and *Bulinus truncatus*, a freshwater snail host in which the parasite reproduce asexually resulting in the production of cercariae that are released in the aquatic environment and actively seek and penetrate the skin of a new vertebrate definitive host.

We hypothesise that for a given abundance of *B. truncatus*, an increase in the specific richness of trematodes using *B. truncatus* as a host at the community scale would lead to an increase in co-infection rates that involves *S. haematobium*. Given that *Schistosoma* species (including *S. haematobium*) tend to be moderate competitors, an increase in the rate of co-infection is expected to reduce the development of *S. haematobium* within their snail hosts and so the total amount of *S. haematobium* cercariae released from the snails into the aquatic environment and ultimately the risk of transmission to their definitive hosts. Moreover, an increase in the abundance of *B. truncatus* would lead to a reduction of the co-infection rates and the associated antagonistic interactions thus ultimately enhancing the risks of schistosomiasis transmission to Humans.

To test these hypotheses, we first built a simple mathematical model to describe (i) how the number of competitive trematodes using *B. truncatus* influence the amount of *S. haematobium* cercariae released in aquatic systems and (ii) how snail density (here carrying capacity) influences co-infections rates and the associated antagonistic effects on the release of *S. haematobium* cercariae at the transmission site level. We confronted this mathematical model to an empirical field work study

conducted in Northern Senegal at 9 previously identified urogenital schistosomiasis transmission sites. We combined traditional malacological and parasitological approaches and next-generation molecular tools to empirically study the effect of the trematodofauna using *B. truncatus* as intermediate host and the local abundance of *B. truncatus* on the transmission of *S. haematobium*.

3. Material and methods

3.1. Mathematical model

2.1.1.Model with one trematode species

We start by modelling the infection of a snail population by a single trematode species. The model tracks the dynamics of the density of miracidia, susceptible and infected snails, and cercariae in the aquatic ecosystem, denoted by the variable *M(t)*, *S(t)*, *I(t)* and *C(t)* respectively. The dynamics are described by the following set of ordinary differential equation :

$$\begin{aligned}
\frac{\mathrm{d}M}{\mathrm{d}t} &= g - eSM - d_M M \\
\frac{\mathrm{d}S}{\mathrm{d}t} &= -eSM - d_S S + f_S S\left(1 - \frac{S+I}{K}\right) \\
\frac{\mathrm{d}I}{\mathrm{d}t} &= eSM - d_I I + f_I I\left(1 - \frac{S+I}{K}\right) \\
\frac{\mathrm{d}C}{\mathrm{d}t} &= sI - d_C C - uC
\end{aligned}$$

These equations use the following parameters: g : input rate of miracidia; e : infection rate of susceptible snails by miracidia ; f_S, f_I : fitness of susceptible and infected snails; K : carrying capacity of snail population; s : shedding rate of infected snails; u : uptake rate of cercariae by mammals; d_M, d_S, d_I , d_C : loss rates of miracidia, susceptible snails, infected snails, cercariae. Because infection reduces fitness and increases mortality, $f_I < f_S$ and $d_I > d_S$. For simplicity we set $f_I = 0$ and $d_S = 0$. Note that the model does not describe the complete trematode life cycle. Rather, the model starts from a given exposure to miracidia (parameter g) and predicts the cercariae density (variable C), or equivalently the uptake of cercariae by mammal hosts (equal to uC). To simplify the study of the model, we look at its equilibrium properties (Additional _File_1_(Figure_S1)).

2.1.2. Model with two trematode species

Next, we consider two trematode species infecting a snail population. We call these two species *1* and *2*. Compared to the previous model, we extend the list of variables as follows: miracidia M_1 and M_2, susceptible S, infected I_1, I_2 and co-infected I_{12}, and cercariae C_1 and C_2. The equations are :

$$\begin{aligned}
\frac{\mathrm{d}M_1}{\mathrm{d}t} &= g_1 - e_1 S M_1 - d_{M_1} M_1 \\
\frac{\mathrm{d}M_2}{\mathrm{d}t} &= g_2 - e_2 S M_2 - d_{M_2} M_2 \\
\frac{\mathrm{d}S}{\mathrm{d}t} &= -e_1 S M_1 - e_2 S M_2 + f_S S\left(1 - \frac{S + \sum I}{K}\right) \\
\frac{\mathrm{d}I_1}{\mathrm{d}t} &= e_1 S M_1 - e_2 I_1 M_2 - d_{I_1} I_1 \\
\frac{\mathrm{d}I_2}{\mathrm{d}t} &= e_2 S M_2 - e_1 I_2 M_1 - d_{I_2} I_2 \\
\frac{\mathrm{d}I_{12}}{\mathrm{d}t} &= e_2 I_1 M_2 + e_1 I_2 M_1 - d_{I_{12}} I_{12} \\
\frac{\mathrm{d}C_1}{\mathrm{d}t} &= s_1 I_1 + s_{1|2} I_{12} - d_{C_1} C_1 - u_1 C_1 \\
\frac{\mathrm{d}C_2}{\mathrm{d}t} &= s_2 I_2 + s_{2|1} I_{12} - d_{C_2} C_2 - u_2 C_2
\end{aligned}$$

The meaning of the model parameters is as before, but now with the indices *1* and *2* referring to the trematode species. For the shedding rates, s_1 is the shedding rate of cercariae of species *1* by a snail infected by species *1* only, whereas $s_{1|2}$ is the shedding rate of cercariae of species *1* by a snail infected by both species. Similar definitions hold for s_2 and $s_{2|1}$. We assume that the two trematode species only differ in their shedding rates. The term "shedding" used in the model reflect the transition from the day when snails become infected to the release of cercariae, and hence also includes the development of the trematodes within the snail hosts. Taking species *1* as a *Schistosoma* species, we model its inferior competitivity by taking $s_{1|2} < s_{2|1}$ (i.e., co-infected snails preferentially shed cercariae of species *2*). For simplicity, we set $s_{1|2} = 0$ and $s_{2|1} = s_2$. In other words, snails that are infected by both species do not shed *Schistosoma* cercariae, and they shed cercariae of the other species at the same rate as a snail not co-infected by *Schistosoma*. Apart from this difference in shedding rates, we assume the two trematode species to be equivalent for all other model parameters (Additional _File_2_&_3_(Figure_S2_and_S3)).

2.1.3.Model with multiple trematode species

The above model can be extended to more than two trematode species. We denote by n the number of trematode species, and assume that snails can be infected by one, two, . . . , all n species. We make the same assumptions as above, that is, all trematode species are assumed to be equivalent apart from their shedding rates. In particular, we consider one *Schistosoma* species and n−1 other trematode species and assume that there is no shedding of *Schistosoma* cercariae in case snails are co-infected with (one or more) other trematode species. We assume that the miracidia input rate per trematode species is independent of diversity, so that the total input rate (i.e., summed over all trematode species) increases proportionally with diversity. In particular, the input rate of *Schistosoma* miracidia is unaffected by the presence of other trematode species. We assume that the total shedding rate (i.e., the shedding rate per infected snail and summed over all shedded trematode species) is independent of the number of species infecting the snail. This implies that the shedding rate per snail infected by a particular trematode species decreases with trematode diversity, as some of these snails are co-infected with other trematode species. This holds especially for snails infected by *Schistosoma*, as co-infection by another trematode species causes the *Schistosoma* shedding rate to drop to zero. An additional file provides an in-depth description of the model equations and our simulations (Additional_File_4_(Supplementary_material), Additional_File_5_&_6)

3.2. Empirical field work

2.2.1.Study sites description and ethical consideration

An empirical fieldwork study was carried out in the region of the Senegal river basin during the dry season in February 2022 (Figure 1). We targeted 9 previously identified *S. haematobium* transmission sites that differ from an ecological point of view (i.e. river, irrigation canal and lake) and hence possibly harbouring different trematode communities. Each of these transmission sites is close to a village where the prevalence and intensity of urogenital schistosomiasis among children was previously reported (18).

The transmission sites close to the villages of Ndiawara (16°35’04”N / 14°50’58”W), Ouali Diala (16°35’56”N / 14°56’10”W), Dioundou (16°35’50”N / 14°53’22”W), Fonde Ass (16°36’20”N / 14°57’38”W) and Khodit (16°35’45”N / 14°56’42”W) are located in the middle valley along a tributary of the Senegal River (“le Doue” river). The transmission site close to the village of Guia (16°35’51”N / 14°55’31”W) is located along an irrigation canal that drains water from the river “le Doue”. The transmission sites nearby the villages of Mbane (16°16'15"N / 15°48'7"W) and Saneinte (16°14'32"N / 15°48'6"W) are located along the east shore of the lake de Guiers. The transmission site close to the village of Lampsar (16°6'34"N / 16°20'58"W) is in an inlet in the lower valley of the Senegal river delta (Figure 1).

2.2.2.eDNA, snails and trematodes field sampling

To assess the abundance of *B. truncatus* and to identify all trematode species present at each of the 9 studied sites as free-living stages, aquatic environmental DNA samples (eDNA) were collected using water filter capsules according to Douchet et al. (2022). Filtrations were carried out from the entire water column along the banks until the filter’s membrane clogged, and the volume filtered at each site was recorded. At each site but Khodit, 1.5L of commercial spring water was filtered following the same protocol as a technical field negative control. Once the water filtrations completed, capsules were drained, filled with 50 mL of Longmire buffer solution to preserve eDNAs, vigorously shaken, and stored at room temperature and at dark until subsequent DNA extraction.

Once the eDNA sampling achieved, all snails found at each sampling site were systematically collected manually or by scooping the grass on the water bench using a colander for about 30 minutes to one hour. Snails were morphologically identified and individualized on well plates filled with dechlorinated water and left to emit trematodes for 2 h in the afternoon under natural sunlight. Cercariae from each emitting snail were collected and transferred onto FTA cards and stored at room temperature until DNA extraction and molecular identification. Coupled with eDNA samples, this approach allows a better characterisation of the trematode community at each transmission site and a

297 quantification of the prevalence of each emitted trematode species among the locally established snail
298 populations including *Bulinus truncatus*. All snails morphologically identified as *B. truncatus* (either
299 emitting or non-emitting) were preserved individually in alcohol until DNA extraction. Subsequent
300 molecular analyses aimed at validating the species attribution of each *B. truncatus* individual, check for
301 the presence and identification of potentially developing trematodes within each snail, refine measures
302 of prevalence of each trematode species, and identify possible coinfections.
303
304 ### 3.3. Experimental infection
305 Twenty six non-emitting snails (although possibly naturally infected by trematodes) sampled at
306 sites Guia, Fonde Ass, Khodit and Saneinte were kept alive and individually exposed to 3 *S.*
307 *haematobium* miracidia for 2 hours in 24 well plates filled with dechlorinated water. The miracidia used
308 for this experimental mollusc exposition were from a homogenised pool of *S. haematobium* eggs of
309 eight children from the Lampsar village provided by the Sen_Hybrid_Invasion project. The 104 exposed
310 snails (i.e. 26 x 4) were maintained and fed *ad libitum* for a period of 1 month and survival was checked
311 daily. At the end of this period, the infection status of each *B. truncatus* was monitored by emission of
312 cercariae once a week until the snails died. To this end, *B. truncatus* were individualized on clean well
313 plates filled with dechlorinated water and stimulated to emit trematodes for 4 h in the afternoon under
314 artificial light. Cercariae released from emitting snails were collected and stored at -20°C until DNA
315 extraction for subsequent molecular identification. All along the experiment, each emitting snail was
316 kept individualised to identify possible trematode species emission shifts through time. At the end of
317 the experiment, all *B. truncatus* individuals (either emitting or non-emitting) were preserved in alcohol
318 individually until DNA extraction.
319

3.4. Molecular approaches

2.4.1.DNA extractions from samples

To extract eDNAs from water filtrates, the Longmire solution contained in each capsule was poured into three 50 ml Falcon tubes as technical replicates. For the field negative controls (i.e. Spring water filtrates), each capsule content was recovered in one 50 ml Falcon tube only. All Falcon tubes were centrifugated at 16,000 g for 20 min and the supernatant was removed. We then collected 0.25 g to 0.5 g of sediment from the pellet from each falcon tube or 500 µL of Longmire remaining at the base of the falcon tube when not enough material was observed. For negative controls, 500 µl of Longmire were systematically retained when discarding the supernatant. This pre-extraction step led to the processing of 35 samples (i.e., 8 negative controls and 3 extraction replicates for each of the 9 environmental samples). Total environmental genomic DNA was extracted from each triplicate and negative controls using the Qiagen's Dneasy PowerSoil Pro Kit following supplier recommendation performing the physical lysis with a MagNA Lyser at a speed of 7,000 for 30s.

DNAs from all cercariae obtained from the field survey, from the exposure experiment and from all *B. truncatus* snails were extracted using the Qiagen DNeasy Blood & Tissue kit. Regarding cercariae preserved onto FTA cards, we manually isolated 0.5 cm in diameter pieces containing the biological material using a sterile punch and placed them in 1.5 ml tubes. Four samples consisting in 0.5 cm in diameter pieces from cards on which ultrapure water had been deposited were also isolated as DNA extraction negative controls. For cercariae preserved in 1.5 ml tubes stored at -20°C, we thawed the tubes and centrifuged them at 20,000g for 10 minutes before removing the supernatant. To obtain extraction and PCR negative controls, we also centrifuged 2 tubes that contained ultrapure water following the same protocol. For *B. truncatus*, we first rinsed the snails with tap water and ground them entirely and individually with a sterile pestle in 1.5 mL tubes. To obtain extraction and PCR negative controls, we also processed 7 assuredly non-infected *B. truncatus* from our laboratory collections in the same way. Moreover, 3 extraction/PCR positive controls were prepared from 3 additional non-infected laboratory *B. truncatus* individuals, to which one *S. haematobium* cercariae from our

laboratory collections was artificially added before DNA extraction processing. These pre-extraction steps led to the processing of 328 samples and 16 controls (i.e., 29 FTA card pieces and 4 associated negative controls, 28 cercaria preserved in alcohol and 2 associated negative controls and 281 *B. truncatus* including 173 *B. truncatus* from the field and 98 from the experimental infection and 3 and 7 associated positive and negative controls). From these samples, we then followed the Tissue protocol as recommended by the supplier applying a 2 h lysis for cercariae and an overnight lysis for snails.

2.4.2. Taxonomical assignment of trematode cercariae by barcoding

To taxonomically assign each cercariae emitted from snail hosts to a trematode species, DNAs extracted from cercariae of each emitting snail were SANGER sequenced at the *28S D2* rDNA gene domain and at the *16S* rDNA gene (19). PCRs were run using the obtained 57 DNAs extracted from cercariae and the 6 negative controls on both markers. PCRs were performed using the GoTaq® G2 Hot Start Polymerase kit of Promega. Each PCR reaction contained Colorless Buffer at 1×, MgCL2 at 1.5 mM, dNTPs at 0.2 mM, primers at 0.4 µM, 1.25 units of GoTaq G2 Hot Start, 2 µl of DNA sample, and ultrapure water for a total PCR reaction volume of 35 µL. For the *28S* marker, the PCR program was used as follows: An initial denaturation step at 94°C for 3' followed by 40 cycles with a denaturation step at 95°C for 30 s, a hybridization step at 56°C for 30 s and an elongation step at 72°C for 30 s. We finally performed a final elongation step at 72°C for 5'. For the *16S* marker, the same PCR program was used, except that 35 cycles were performed, the hybridization temperature was 54°C and the elongation time was 15 s. Ten microliters of the resulting PCR products were migrated on a 2% agarose gel for 20' at 135 V and revealed using a Vilber Infinity 1000 imaging system. Each individual PCR product from the 57 cercariae DNA extracts that displayed an expected theoretical size was then sequenced in both the forward and reverse directions on an ABI 3730xl sequencer at the GenoScreen platform (Lille, France). The sequences generated were aligned, trimmed, and taxonomically assigned with a MEGABLAST analysis for the *28S* and the *16S* markers. For the taxonomical assignment of each

cercariae, the common taxonomic rank to all hits above 96% of identity over 97% of coverage were kept.

2.4.3.Molecular taxonomic validation of B. truncatus and infection diagnostic

The 271 *Bulinus* spp. sampled in the field from which total DNA was extracted were diagnosed to verify that they belonged to the species *B. truncatus* using a molecular diagnosis by LAMP based on the internal transcribed spacer (ITS2) *B. truncatus* species-specific (20). DNAs extracted from *Bulinus* spp. were first diluted at 1/100e and a negative control consisting in a 1/100e diluted DNA extraction from *Bulinus globosus* was used. Each LAMP reaction contained an Isothermal Amplification Buffer II reaction buffer [20 mM Tris–HCl, 10 mM (NH4)2SO4, 150 mM KCl, 2 mM MgSO4, 0. 1% Tween 20, pH 8.8 at 25 °C (New England Biolabs, UK)] at 1X, additional MgSO4 at 3 mM, dNTP at 1.0 mM, internal primers FIP and BIP at 1.2 µM, external primers F3 and B3 at 0.2 µM, LOOP primers LB and LF at 0.4 µM, 1 U of Bst 2.0 WarmStart DNA polymerase (New England Biolabs, UK), 1 µl of DNA sample, and ultrapure water for a total reaction volume of 10 µL as described in Blin et al., 2023. Reaction were performed in a thermocycler at 63 °C for 45 min followed by an enzyme inactivation phase at 80 °C for 5 min. Result visualizations were done using a final point visual detection of fluorescence after adding 1 µl of 1:50 diluted 10,000× concentration of SYBR Green (Invitrogen) (green: positive=*B. truncatus* species; orange: negative=other than *Bulinus* species).

To detect trematodes within the tissues of each *B. truncatus*, we used the 16S rDNA gene metabarcode initially developed to characterize trematode communities from eDNA (19) as a molecular diagnostic tool. PCRs were performed using the GoTaq® G2 Hot Start Polymerase kit of Promega following the same PCR condition as described previously [see section 2.4.2], except that reactions were performed in a final volume of 10 µl and using 2 µL of DNA extracted from *Bulinus* spp. diluted 1:100. Amplification success was assessed visually by migrating the ten microliters of the resulting PCR products on a 2% agarose gel for 20’ at 135 V and revealed using a Vilber Infinity 1000 imaging system.

2.4.4.Trematodes-specific MiSeq sequencing on the infected B. truncatus individuals and on the eDNA to characterize trematode communities

A total of 137 positive *16S* metabarcoding NGS libraries were prepared following the Illumina two-step PCR protocol, using the Trem_16S_F1 and the Trem_16S_R2 primers set up with Illumina adapters using 2 µL of eDNA or DNA extracted from *B. truncatus* diluted at 1:100e as previously described (19). Libraries were sent and paired-end sequenced (2 × 250 bp) on an Illumina MiSeq™ at the BioEnvironnement platform (University of Perpignan Via Domitia, France). Three samples were eliminated from the analyses of the MiSeq sequencing because they did not meet the defined threshold of 25,000 sequences required to normalise samples by rarefaction (i.e., one non emitting *B.truncatus* from the site Ouali Diala, and two PCR duplicates from water eDNA).

2.4.5.Abundance of B. truncatus determination by ddPCR on eDNA

Abundance of *B. truncatus* at each study site was assessed by digital droplet PCR (ddPCR) from eDNA sampled by water filtration according to (21). We first pooled the eDNA extraction triplicates from each of the 9 sampling sites. As negative control, we also pooled DNAs extracts from one individual of each mollusc species sampled during the field work in addition to DNAs extracts from 3 *B. truncatus* sister species (i.e., *B. globosus*, *B. senegalensis* and *B. umbilicatus*) from our laboratory collection. As positive control, we used a DNA extract at 0.01 ng/µL from one *B. truncatus* from our laboratory collection. We ran ddPCRs using the TaqMan technology on a QX200 AutoDG Droplet Digital System (BioRad) at the Bio-environnement platform (Perpignan, France). We used the Btco2F (5' ATTTTGACTTTTACCACCAT 3') and Btco2R (5' GATATCCCAGCTAAATGAAG 3') primers combined with the FAM-labelled probe Btco2P (5' TCGAAGGAGGGGTTGGAACAGG-FAM 3'). ddPCR reactions were performed using the BioRad ddPCR Supermix for Probes (No dUTP). Each ddPCR reaction contained the BioRad MIX at 1X, primers at 0.5 µM, probes at 0.25 µM, 7 µL of eDNA template or 2 µL of control DNAs templates, and ultrapure water for a total ddPCR reaction volume of 20 µL. After the droplets

generation, the ddPCR program was used as follows: An enzyme activation step at 95°C for 10 min followed by 40 cycles with a denaturation step at 94°C for 30s and an annealing/extension step at 60°C for 60s, with a ramp setting to 2°C/s. We finally performed an enzyme deactivation step at 98°C for 10'. The resulting fluorescence signal were analysed using QuantaSoft software V1.7 (BioRad). A signal was considered positive (i.e., with the presence of *B. truncatus* DNA) if at least one positive droplet was detected and if the positive droplet displayed the same order of fluorescence magnitude as the positive droplet obtained from a ddPCR positive control.

3.5. Data analysis

2.5.1. Characterisation of trematode communities present in the water and exploiting B. truncatus populations

The resulting amplicon sequence datasets from the MiSeq sequencing was processed using the Find Rapidly OTUs with Galaxy Solution (FROGS) (22) according to (19). Briefly, the produced datasets were pre-processed by filtering out the sequences to keep amplicon sizes from 150 to 400 nucleotides. The remaining sequences were next clustered into operational taxonomic units (OTUs) using the swarm algorithm and using denoising and an aggregation distance of three (23). The resulting dataset was filtered out for chimeras using VSEARCH (24). Singletons and underrepresented clusters (i.e., clusters whose number of sequences were <0.1% of the total number of sequences) were removed. Lastly, we conservatively considered that a given OTU was present in a library if its number of sequences was >0.1% of the total number of sequences in this library.

Each OTU was next assigned to a taxonomic level (either a species or a genus) using a two-step BLAST affiliation process. The first BLAST analysis was computed using the standalone blastn program contained in the *BLAST+* package and a custom trematode sequence database containing 174 sequences (82 sequences from the NCBI database and 92 sequences from a custom internal trematode sequence database including the sequences obtained from the amplicons generated by the SANGER sequencing on cercariae, see section 1.1.7). The second BLAST analysis was performed using the online

MEGABLAST tool and based on the nr database without restricting parameters to achieve affiliation of OTUs that could not be assigned in the first BLAST analysis. OTUs were assigned to a species if the sequences presented a minimal blast coverage of 97% and a pairwise identity above 99% with the affiliated sequence. OTUs were assigned to a genus if the sequences presented a minimal blast coverage of 97% and a pairwise identity above 96% with the affiliated sequence. OTUs that could not be assigned to a species or genus were assigned to a higher taxonomic rank using a clustering method based on the pairwise genetic distances between the OTUs and the same set of 174 sequences as above. We then aligned these sequences with T-Coffee on EMBL-EBI (25) and built a neighbor joining phenetic tree based on the percentage of nucleotide differences from the obtained alignment using Jalview version 2.11.1.4 (26) for visualization.

Subsequent analyses were then performed on R version 4.3.1. Each library was normalized by rarefaction at 25,000 reads using the package *Vegan* version 2.6-4. The trematode composition, specific richness and relative abundance in each *B. truncatus* individual or water sample was next assessed using the package *phyloseq* (27). We assessed the co-infection rates among infected *B. truncatus* and the number of trematode species involved in each co-infection. We also tested whether parasite aggregation at the *B. truncatus* individual scale occurred by comparing the observed distribution of trematode species within hosts with a theoretical distribution following a negative binomial distribution using a Chi-square test.

2.5.2.Abundance of B. truncatus at each sampling site

The *B. truncatus* eDNA copy number per liter of filtered water (C_L) estimated from the ddPCR results was used as a proxy of the abundance of *B. truncatus* at each sampling site. C_L was calculated from the following equation (21):

$$C_L = \frac{\frac{C_{rdd} * V_e}{V_r}}{V_w}$$

With C_L: the number of eDNA c/L for the amplified sample ; C_{rdd}: the copy number per reaction volume ; V_e: the total volume of eluted DNA after extraction ; V_r: the volume of extracted DNA used for ddPCR reaction ; and V_w: the total volume of filtered water.

2.5.3.Assessing the link between the relative abundance of Schistosoma quantified in co-infected B. truncatus and the emission status of Schistosoma cercariae by co-infected B. truncatus

To determine whether the dominance status of *Schistosoma* species in terms of percentage of reads of these species within *B. truncatus* individuals co-infected with other trematode species can be used as a proxy of *Schistosoma* cercarial release, we performed a generalised linear model (GLM) using the package *stats* version 4.3.1 implemented in R. A subset from our dataset that contained 14 *B. truncatus* individuals from the field work and from the experimental infection were used for this analysis. These individuals were all emitters, co-infected and harboured at least *S. haematobium* or *S. bovis*. We set the emission status (i.e. 0 and 1: non-emitting and emitting *Schistosoma sp.*) as the dependent variable and the relative abundance of the respective *Schistosoma* species in terms of percentage of sequences compared to the other co-infecting trematode species as explanatory variable. The model was built assuming a quasibinomial distribution of the data.

2.5.4.Effect of trematode species richness, co-infection rates and host abundance on the average dominance of schistosomes

To test our initial hypotheses, we ran three independent generalized linear mixed models (GLMM) using the package *lme4* version 1.1-33 implemented in R to infer the effect of (i) the overall trematode species richness using *B. truncatus* as an intermediate host at the population level, (ii) the co-infection rate among the infected *B. truncatus* snails, and (iii) the abundance of *B. truncatus;* on the average dominance of schistosomes within host populations (i.e., the relative abundance of *S. haematobium* or *S. bovis* in terms of percentage of sequences compared to the other co-infecting

trematode species). Only naturally infected *B. truncatus* from the field were considered in this analysis, excluding *B. truncatus* from the experimental infection. To account for pseudo-replication, we set the sampling site as a random factor. For these analyses, the co-infection rate variable was transformed into two categories (i.e. <50% and >50% co-infection among infected individuals). The *B. truncatus* abundance variable was also transformed into two categories (i.e. <200 and >200 C_L) to compare sites at low and medium to high density according to Mulero et al. 2020. The variable "trematode species richness" (that uses *B. truncatus)* was coded as the number of trematode species other than *Schistosoma* which use local *B. truncatus* population at the site level. We assumed a binomial error term in the three models tested.

4. Results

4.1. Mathematical model

Our mathematical model indicates that, for a fixed carrying capacity of *B. truncatus* (either small or large), a local increase in the number of competitive trematode species using *B. truncatus* as intermediate host leads to an increase in the co-infection rate within individual snails and ultimately to a decrease in the number of *S. haematobium* cercariae produced (Figure 2). Moreover, an increase of carrying capacity of *B. truncatus* leads to a decrease of the proportion of co-infected snails and an increase in the proportion of *Schistosoma* cercariae (Figure 2). In other words, an increase in the number of competitive trematode species using *B. truncatus* as intermediate host can reduce the production of *S. haematobium* cercariae through antagonist interactions and particularly so when *B. truncatus* populations are small. An increase in *B. truncatus* populations mitigates the effect of antagonist interactions by reducing the coinfection rate among snail hosts.

4.2. Empirical field study

Except *Bulinus forskalii* that was recorded at low abundance at 3 sites (i.e., Ouali Diala, Guia and Khodit), *B. truncatus* was the only *Bulinus* species found during the field survey. The species identity of the 271 snails initially identified as *B truncatus* based on their morphology, and from which total DNA was extracted, was validated by the LAMP-based diagnostic tool. The abundance of *B. truncatus* among sites assessed based on digital PCRs results varied from 41 to 2176 eDNA copies per litre filtered (Table 1).

In addition to *S. haematobium* and its sister species *S. bovis*, we identified 9 trematode species associated with *B. truncatus* populations. Nine trematode species were sampled during our fieldwork (Figure 3), and two additional trematode species were detected during the monitoring of experimentally infected *B. truncatus* individuals, including *Apharyngostrigea pipientis* and an *Echinostoma* species that we could not determine at the species level (Additional_File_7_(Table S1)). An additional species (i.e. Paramphistomoidea sp. 3) was found associated to two *B. truncatus* individuals from the experimental infection (Additional_File_7_(Table S1)) under the appearance of cysts attached on their shell (despite washing) but with no apparent shedding from snails. The number of trematode species other than S*. haematobium* and *S. bovis* using the *B. truncatus* population at each site varied from 0 to 4 species and the co-infection rates among infected individuals from 0 to 100% (Figure 3; Table 1). The distribution of the number of trematode species per *B. truncatus* individual from the field did not differ significantly from a theoretical negative binomial distribution hence indicating a classical parasite aggregation pattern in *B. truncatus* populations (Additional_File_8_(Figure S4)).

Apart from the *B. truncatus* population, a total of 20 trematode species were detected from the water samples, 7 of which were also detected in *B. truncatus* while 2 trematodes detected in *B. truncatus* were not detected from the water samples. *Schistosoma haematobium* and/or *S. bovis* were detected from eDNA water filtrates at 5 among the 9 targeted sites (Additional_File_9_(Table S2)).

549 Table 1 : Volume of water filtered, abundance of *B. truncatus*, molecular prevalence of *Schistosoma* in
550 *B. truncatus* populations, specific richness of trematodes that use *B. truncatus* populations as a host
551 (other than *Schistosoma*), co-infection rate among infected snails, and average number of reads
552 attributed to *Schistosoma* among snails infected by *Schistosoma*, per site.

Site	Volume of water filtered (L)	*B. truncatus* abundance (DNA copies / litre of filtered water)	Molecular prevalence of *Schistosoma* in *B. truncatus*	Trematode richness using *B. truncatus* as host other than *schistosoma*	Rate of co-infections among infected individuals	Average % of *Schistosoma* reads in *B. truncatus* individuals infected by *Schistosoma*
Ndiawara	6.5	78	0,0	4	0.33	00.00
Ouali_Diala	10	41	2,3	3	0.83	34.94
Guia_canal	5	61	23,8	3	1.00	20.67
Dioundou	10	75	0,0	0	NA	00.00
Fonde_Ass	10	57	0,0	1	0.00	00.00
Khodit	5	333	29,4	1	0.20	99.74
Mbane	2.8	104	0,0	0	NA	00.00
Saneinte	3.5	2176	8,3	2	0.25	50.15
Lampsar	18	58	6,3	1	0.25	80.63

553

554 During all the molecular analysis steps, all the negative and positive controls behaved as
555 expected. The prevalences of trematodes including that of schistosomes in *B. truncatus* populations,
556 assessed from our molecular diagnostic approach, were higher than the prevalences measured by the
557 emission method only (Additional_File_10_(Table S3)).

558 Overall the emitting *B. truncatus* infected by *Schistosoma* species and by at least one other
559 species of trematode, *Schistosoma* species are shed only when they are dominant in terms of relative
560 sequenced reads abundance compared to that of the other co-infecting trematode species
561 (Additional_File_11_(Figure_S5)). Moreover, in all but one case, the haplotype of the trematode
562 species emitted corresponds to the most abundant haplotype obtained from NGS sequencing in co-
563 infected *B. truncatus* (Figure 3; Additional_File_7_(Table_S1)).

Both the local richness of trematode species exploiting *B. truncatus* population (p-value = 0.029; AIC = 14.9) and the coinfection rate (p-value = 0.020, AIC = 17.4) have a significant negative effect on *Schistosoma spp.* dominance based on our GLMM models. Conversely, the local abundance of *B. truncatus* positively and significantly affects *Schistosoma spp.* dominance (p-value = 0.047, AIC =20.1). Based on the comparison of AICs obtained from the three statistical models ran, the model accounting for the local richness of trematode species exploiting *B. truncatus* population is the best supported which strengthens the idea that this factor is the most important in explaining *Schistosoma spp.* dominance. At site level, the co-infection rate among infected snails tended to increase when the total trematode species richness exploiting the *B. truncatus* population increased, although the correlation was not significant (Table 1). Conversely, this same co-infection rate tended to decrease when the abundance of *B. truncatus* increased (Table 1).

5. Discussion / Conclusion

Understanding the ecological mechanisms that drive the circulation of parasites is primordial to better identify transmission site, better assess transmission risks and guide strategies to fight against parasites and the associated diseases while preserving the integrity of the socio-ecosystem health (28,29). In this context, the ecology of transmission of *Schistosoma* species that are involved in bilharziasis forms associated to humans and to a lesser extent to animals, and the impact of human activities on the circulation of these parasites have received considerable attention and particularly so in the last decade (30,31).

The presence and abundance of compatible snail hosts in a given system are determining factors in the establishment and circulation of *Schistosoma* parasites, particularly as they modulate the rate of contact between the parasites released into the system and the hosts (i.e. encounter filter; (32,33)).

In the present study we theoretically and empirically show that reducing snail host abundance also hamper the circulation of *S. haematobium* indirectly by promoting snail co-infections with other

potentially more competitive trematode species and hence a reduction of *S. haematobium* cercariae produced by local *B. truncatus* populations. This competition effect on the circulation of *S. haematobium* becomes negligeable when *B. truncatus* populations are abundant. In other word, the size of local snail host populations for parasites of the genus *Schistosoma* may predict the risk of transmission for definitive vertebrate hosts including humans because it determines not only the encounter filter, but also modulates the 'competence' (*sensu largo*) of the locally established snail hosts by influencing their probability of being co-infected with other competitive trematode species (i.e. compatibility filter; (32,33)). This result is based on the generally acknowledged assumption that *Schistosoma* species are bad to moderate competitors (13,15). Although we could not empirically assess the hierarchical rank of competitive ability of each trematode detected in *B. truncatus* populations during our field survey we can expect that at least some of these outcompete or even predate *S. haematobium*, particularly those known to produce rediae during their intra-mollusc parasitic stage such as *Petasiger* sp.

Coinfections are rarely observed in the field which could suggest that the effect of the resulting antagonistic interactions between co-occurring parasites could be of relatively weak importance. We here argue that coinfections are likely to be generally underestimated. In particular, we believe that traditional techniques to detect trematodes that develop within their snail hosts via dissection or induced cercarial emission, might lead to an underestimation of coinfection rates. This might be particularly true when *Schistosoma* species are present in the form of degraded, or even invisible traces, resulting from competition or predation induced by other trematodes species. In this regard, high throughput next generation sequencing approaches such as metabarcoding used in this study appear promising to better assess coinfection rates and overall trematode communities within natural snail host populations (34). These metabarcoding approaches are the 'interspecific equivalent' of the genotyping approaches that have revealed the initially unsuspected intraspecific diversity of parasites within their hosts in the past decades (35–37).

615 Our model also points toward the fact that the intensity of such within-snail host competitive
616 effects on *S. haematobium* cercariae production increases with the number of trematode species using
617 the targeted *B. truncatus* population. This theoretical result is also supported by our empirical study. It
618 is interesting to note that the presence of a single highly competitive trematode species at high
619 abundance at a given transmission site and using a given population of *B. truncatus*, would locally result
620 in a similar decrease in the amount of cercariae of *S. haematobium* co-occurring at this site. In fact,
621 biological control strategies based on the dissemination of farmable competitive trematode species
622 that use snail vectors of parasites of the genus *Schistosoma* at active transmission sites have been
623 proposed in the past (38). However, this situation, if not artificially maintained, is generally uncommon
624 *in natura*. Conversely, several studies, including ours, indicate that parasites of the genus *Schistosoma*
625 naturally cooccur frequently with several trematode species that use *Schistosoma* snail intermediate
626 hosts in the field (13). Since trematode species composition is determined by the composition of
627 vertebrate communities locally established either temporary or permanently (39), maintaining
628 vertebrate host diversity that supports trematode diversity in local snail populations could help to
629 reduce the transmission of *Schistosoma* species. For this effect to be sustainable over time, the
630 trematodes released by their vertebrate definitive hosts must complete their entire life cycle locally,
631 which also implies the maintenance of other compartments of biodiversity, including numerous
632 vertebrates (e.g. fish, amphibians) and invertebrates (e.g. aquatic insects). Unfortunately, our
633 ecological knowledge of most trematodes is sorely lacking, and their life cycles are still poorly
634 documented. Huge works remain to be done to characterise both the fauna involved in supplying
635 trematodes that interact with species of the genus *Schistosoma* in their intermediate hosts, and the
636 fauna involved in maintaining the local life cycles of these trematodes. We believe that the newly
637 available metabarcoding tools such as used in the present study (19,34,40), combined with the
638 implementation of large and well documented sequence datasets, provide a promising avenue to
639 characterise trematode life cycles.

Our study suggest that maintaining high levels of biodiversity in freshwater aquatic ecosystems could help reducing the transmission of parasites of the genus *Schistosoma*. Several synergetic effects of biodiversity could reduce the circulation of these parasites (29). For instance, several organisms including fish crustaceans and oligochaetes can predate free-living stages of *Schistosoma* parasites and hence reduce their abundance and therefore the risk of infection for mammals (41). However, the negative effect of local biodiversity on the circulation of *Schistosoma* species is all the greater when host snail populations are small. Eradication of snail populations that host *Schistosoma* species is generally considered as the best alternative to fight against schistosmiasis and is one of the recommendations of the WHO (42). Although this strategy is theoretically efficient at short term, its deployment on a large spatial and temporal scale is questionable for several reasons. First, the molluscicides commonly used and spread into aquatic ecosystems are sometimes poorly accepted by Human communities using these ecosystems for water supply. Second, the effects of molluscicides on the overall biodiversity associated with aquatic environments are still poorly understood (43–46). Finally, while these products are efficient against snail communities established locally, they do not prevent the recolonization of these species after treatment. In this context, and alternatively to the application of chemical-based molluscicide, several environment-based strategies aiming at reducing snail hosts abundance while providing services to local human populations are emerging such as the introduction of edible predators of snail hosts (e.g. crayfish) or the extraction of specific aquatic plants that serve as refuge for aquatic snails to produce compost, (47,48). Combined with massive drug administration campaign in human populations and the application of the WASH protocol (42), these guided sustainable strategies of reduction or eradication of aquatic snail host populations that constitute intermediate hosts for *Schistosoma*, generally lead to an important reduction in the rate of re-infection and prevalence of schistosomiasis in neighbouring human populations (49). We here argue that the implementation of sustainable strategies to preserve the biodiversity associated with aquatic ecosystems could be an additional and potentially low-cost means of significantly reducing the prevalence and intensity of bilharziasis, without necessarily eliminating totally host snail populations.

These strategies are in line with the need for concrete applications of the One Health concept, particularly recently applied in the case of aquatic parasites including schistosomes (50), and the objectives established by the WHO to reduce the prevalence and intensity of schistosomes below a threshold below which these diseases can no longer be considered a public health problem (42).

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Figures in main text

Figure 1: Satellite map of the studied area in Northern Senegal.

This map indicates the location of the 9 targeted sites (black dots) and the name of the nearby 9 villages associated to these *S. haematobium* transmission sites.

Figure 2 : Co-infection ratio, *Schistosoma* prevalence, and *Schistosoma* cercariae ratio, for three values of the carrying capacity.

The co-infection ratio is the ratio between the number of co-infected *B. truncatus* and the total number of infected *B. truncatus*. The *Schistosoma* cercariae ratio is the ratio between the number of *Schistosoma* cercariae and the total number of cercariae. The dashed purple line indicates the *Schistosoma* cercariae ratio in case the *Schistosoma* shedding rate is the same as for the other trematode species. The reduction

of the *Schistosoma* cercariae ratio (difference between full and dashed purple line) is due to coinfections and to the inferior competitivity of *Schistosoma*. The carrying capacity *K* differs by factors of 10 of the snail population.

Figure 3: Relative abundance of trematode species in percentages of obtained sequenced reads exploiting infected *B. truncatus* individual.
Infected snails (in columns) are grouped by site. The black dots correspond to the species of trematode emitted by the emitting snails (confirmed by SANGER sequencing and BLAST assignation).

7. Additional files

File name : Additional_File_1_(Figure_S1)
File format : .png
Title and description of the data : Equilibrium changes of density of miracidia, cercariae, susceptible and infected snails, as a function of the carrying capacity (*K*) of the snail population in the case of one trematode species infect the snail population.

File name : Additional_File_2_(Figure_S2)
File format : .png
Title and description of the data : Equilibrium changes of density of total miracidia and total cercariae of two trematodes species including one with a lowest competitive capacity (i.e., *Schistosoma*), and of density of susceptible and infected snails, as a function of the carrying capacity *K* of the snail population.

File name : Additional_File_3_(Figure_S3)
File format : .png
Title and description of the data : Equilibrium changes of co-infection ratio and *Schistosoma* cercariae density ratio as a function of the carrying capacity (*K*) of the snail population in the case of two trematodes species infecting the snail population. Note that without co-infection the ratio of *Schistosoma*

834 cercariae would be equal to 1/2. The deviation observed here is due to, and increases with, the co-
835 infection ratio.
836
837 File name : Additional_File_4_(Supplementary_material)
838 File format : .doc
839 Title and description of the data : Supplementary_material providing an in-depth description of the
840 model equations and simulations
841
842 File name : Additional_File_5_divpara_main.m
843 File format : MatLab (.m) https://fr.mathworks.com/products/matlab.html
844 Title and description of the data : MatLab script to generate Figure 2
845
846 File name : Additional_File_6_divpara_rhs.m
847 File format : MatLab (.m) https://fr.mathworks.com/products/matlab.html
848 Title and description of the data : MatLab functions to evaluate the right-hand side of the model
849 equations
850
851 File name : Additional_File_7_(Table S1)
852 File format : .xls
853 Title and description of the data : OTUs table assigned as trematodes of *B truncatus* from the
854 experimental infection positive to the trematode diagnostic
855
856 File name : Additional_File_8_(Figure_S4)
857 File format : .png

Title and description of the data : Number of *B. truncatus* individuals non infected, and infected by one to 4 parasites species. Observed data are shown as bars. The negative binomial theoretical distribution is represented by the black dots. The Chi2 test shows no statistical difference between the observed distribution and the theoretical distribution suggesting a parasites aggregation in host populations (p-value = 0.9985).

File name : Additional_File_9_(Table S2)

File format : .xls

Title and description of the data : OTUs assigned as trematodes found in water filtrations and in *B truncatus* populations

File name : Additional_File_10_(Table S3)

File format : .xls

Title and description of the data : Prevalence of *Schistosoma* and trematodes in the different populations of *B. truncatus*

File name : Additional_File_11_ (Figure S5)

File format : .png

Title and description of the data : Cercariae emission of *S. haematobium* or *S. bovis* as a function of their relative abundance in case of co-infection with one or more other trematode species. Only *B. truncatus* snails emitters infected by one species of *Schistosoma* and by at least one other species of trematode were taken into account in this analysis (i.e. 14 snails). intra-mollusc parasitic stages and cercariae of schistosomes are illustrated in red. Purple represents the intra-mollusc parasitic stages and cercariae of other co-infecting trematode species.


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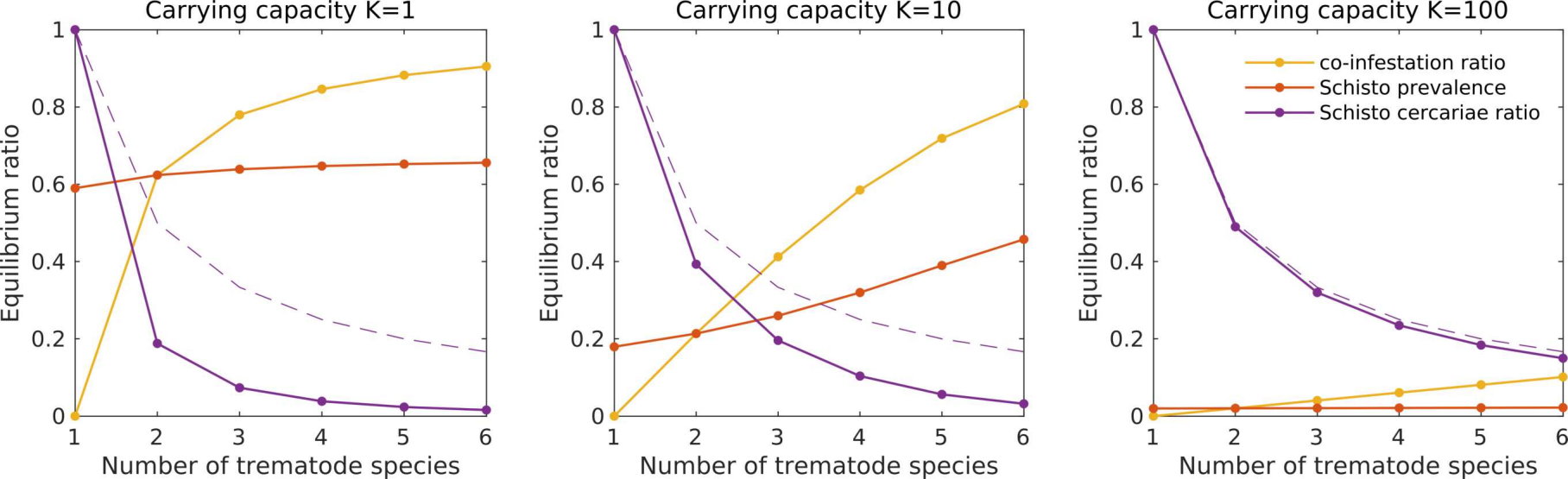
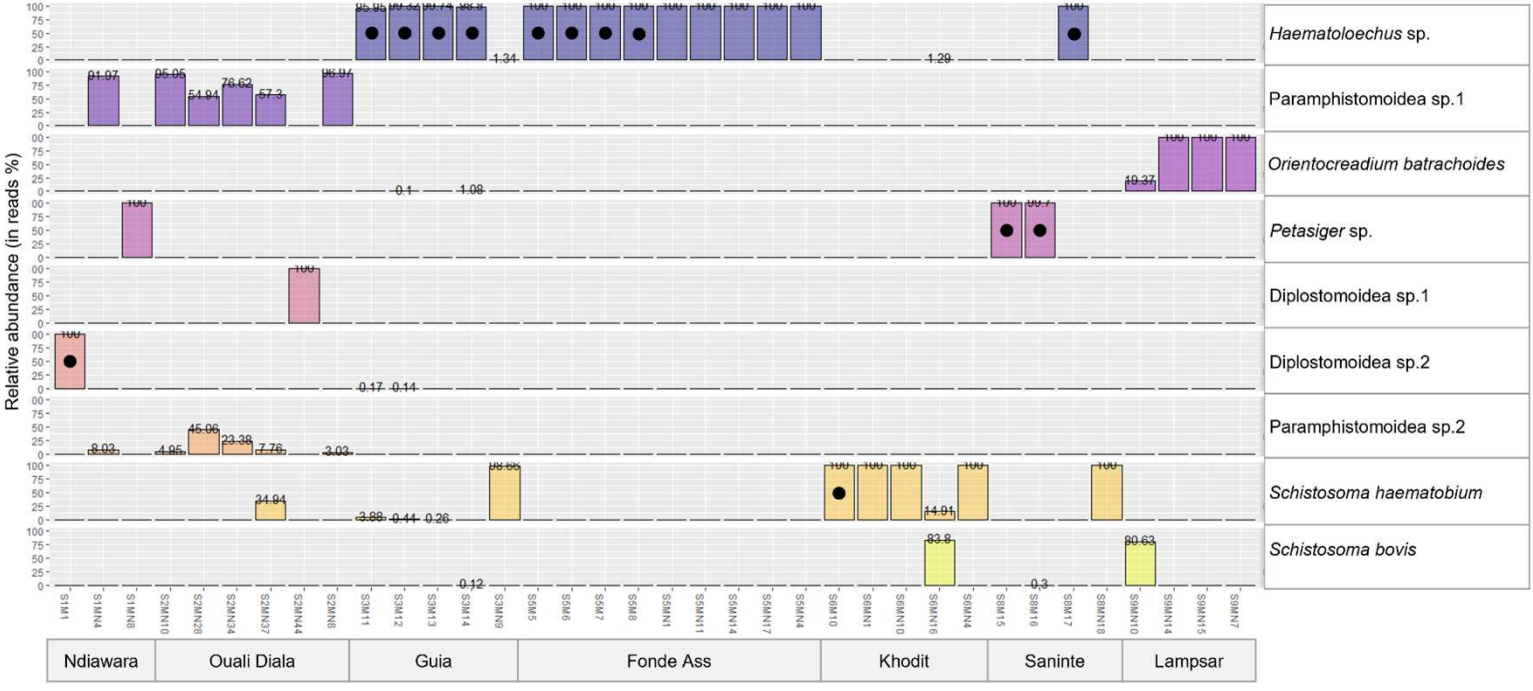

Carrying capacity K=1
Carrying capacity K=10
Carrying capacity K=100
Equilibrium ratio
Number of trematode species
co-infestation ratio
Schisto prevalence
Schisto cercariae ratio
0
0.2
0.4
0.6
0.8
1
1
2
3
4
5
6


Relative abundance (in reads %)
Haematoloechus sp.
Paramphistomoidea sp.1
Orientocreadium batrachoides
Petasiger sp.
Diplostomoidea sp.1
Diplostomoidea sp.2
Paramphistomoidea sp.2
Schistosoma haematobium
Schistosoma bovis
S1M1
S1M4
S1M8
S2M10
S2M28
S2M34
S2M37
S2M44
S2M8
S3M11
S3M12
S3M13
S3M14
S3M9
S5M5
S5M6
S5M7
S5M8
S5M1
S5M11
S5M14
S5M17
S5M4
S6M10
S6M1
S6M10
S6M16
S6M4
S8M15
S8M16
S8M17
S8M18
S9M10
S9M14
S9M15
S9M7
Ndiawara
Ouali Diala
Guia
Fonde Ass
Khodit
Saninte
Lampsar

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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- AdditionalFile2FigureS2.png
- AdditionalFile3FigureS3.png
- AdditionalFile4Supplementarymaterial.docx
- AdditionalFile5divparamain.m
- AdditionalFile6divpararhs.m
- AdditionalFile7TableS1.xlsx
- AdditionalFile8FigureS4.png
- AdditionalFile9TableS2.xlsx
- AdditionalFile10TableS3.xlsx
- AdditionalFile11FigureS5.png