



# Environmental DNA for improved detection and environmental surveillance of schistosomiasis

Mita E. Sengupta<sup>a,1</sup>, Micaela Hellström<sup>b,c</sup>, Henry C. Kariuki<sup>d</sup>, Annette Olsen<sup>a</sup>, Philip F. Thomsen<sup>b,e</sup>, Helena Mejer<sup>a</sup>, Eske Willerslev<sup>b,f,g,h</sup>, Mariam T. Mwanje<sup>i</sup>, Henry Madsen<sup>a</sup>, Thomas K. Kristensen<sup>a</sup>, Anna-Sofie Stensgaard<sup>j,2</sup>, and Birgitte J. Vennervald<sup>a,2</sup>

<sup>a</sup>Department of Veterinary and Animal Sciences, University of Copenhagen, DK-1870 Frederiksberg, Denmark; <sup>b</sup>Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen, DK-1350 Copenhagen K, Denmark; <sup>c</sup>Aquabiota Water Research, SE-115 50, Sweden; <sup>d</sup>Department of Microbiology and Parasitology, Kenya Methodist University, 60200 Meru, Kenya; <sup>e</sup>Department of Bioscience, University of Aarhus, DK-8000 Aarhus C, Denmark; <sup>f</sup>Department of Zoology, University of Cambridge, CB2 3EJ Cambridge, United Kingdom; <sup>g</sup>Human Genetics Programme, Wellcome Trust Sanger Institute, Hinxton, SB10 1SA Cambridge, United Kingdom; <sup>h</sup>Danish Institute for Advanced Study, University of Southern Denmark, DK-5230 Odense M, Denmark; <sup>i</sup>Neglected Tropical Diseases Unit, Division of Communicable Disease Prevention and Control, Ministry of Health, Nairobi, Kenya; and <sup>j</sup>Center for Macroecology, Evolution and Climate, Natural History Museum of Denmark, University of Copenhagen, DK-2100 Copenhagen, Denmark

Edited by Andrea Rinaldo, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, and approved March 18, 2019 (received for review September 21, 2018)

Schistosomiasis is a water-based, infectious disease with high morbidity and significant economic burdens affecting >250 million people globally. Disease control has, with notable success, for decades focused on drug treatment of infected human populations, but a recent paradigm shift now entails moving from control to elimination. To achieve this ambitious goal, more sensitive diagnostic tools are needed to monitor progress toward transmission interruption in the environment, especially in low-intensity infection areas. We report on the development of an environmental DNA (eDNA)-based tool to efficiently detect DNA traces of the parasite *Schistosoma mansoni* directly in the aquatic environment, where the nonhuman part of the parasite life cycle occurs. This is a report of the successful detection of *S. mansoni* in freshwater samples by using aquatic eDNA. True eDNA was detected in as few as 10 cercariae per liter of water in laboratory experiments. The field applicability of the method was tested at known transmission sites in Kenya, where comparison of schistosome detection by conventional snail surveys (snail collection and cercariae shedding) with eDNA (water samples) showed 71% agreement between the methods. The eDNA method furthermore detected schistosome presence at two additional sites where snail shedding failed, demonstrating a higher sensitivity of eDNA sampling. We conclude that eDNA provides a promising tool to substantially improve the environmental surveillance of *S. mansoni*. Given the proper method and guideline development, eDNA could become an essential future component of the schistosomiasis control tool box needed to achieve the goal of elimination.

schistosomiasis | *Schistosoma mansoni* | environmental DNA | snails | elimination

Schistosomiasis is a debilitating snail-borne disease caused by parasitic worms (blood-flukes) of the genus *Schistosoma* (Fig. 1) (1). It is estimated that at least 250 million people globally are infected, and a total of 779 million people in 74 countries are at risk for infection (2, 3). Schistosomiasis is considered a neglected tropical disease (NTD), and >80% of the infected people live in sub-Saharan Africa (2). Since the turn of the new millennium, the global control strategy has focused on targeted mass drug administration (MDA) programs, leading to reduced worm infections and general improvements in human health (4). However, the focus in schistosomiasis control has now shifted from morbidity control toward transmission-focused interventions (5, 6), as the latest WHO roadmap for disease control aims for elimination (4, 7). This entails a complete interruption of transmission in the environment and thus emphasizes the need for improved environmental surveillance (6). Areas with several years of MDA are expected to have low levels of parasite transmission, but continued MDAs alone are unlikely to interrupt parasite transmission.

Furthermore, as infection levels decrease in the human population with ongoing treatment, assessing transmission risk by detecting egg-patent infections in humans becomes less effective (8). Thus, the development and implementation of supplementary environmental surveillance methods to effectively identify the presence of schistosome larval stages in the aquatic intermediate host snails or directly in aquatic environments (or semiaquatic environments as in the case of *Schistosoma japonicum*) is becoming crucial (6, 9).

As the schistosome parasites critically depend on freshwater snails to complete their life cycle (Fig. 1), environmental surveillance has until now been centered on snail-based surveys. This involves collection and correct identification of host snails followed by light-induced shedding of cercariae from each individual snail (10). Such snail surveys are cumbersome and require substantial specific training and expertise. Furthermore, the sensitivity of this approach is generally low, since only a few

## Significance

Accurate detection and delineation of schistosomiasis transmission sites will be vital in ongoing efforts to control and ultimately eliminate one of the most neglected tropical parasitic diseases affecting >250 million people worldwide. Conventional methods to detect parasites in the environment are cumbersome and have low sensitivity. We therefore developed an environmental DNA (eDNA)-based method for schistosome detection in aquatic environments. Aquatic eDNA showed higher sensitivity than conventional snail surveys. We conclude that eDNA is a promising noninvasive and sensitive tool for environmental surveillance of schistosomiasis transmission. As the efforts and aims to control the disease are transitioning toward complete transmission interruption, this could be the robust and cost-effective surveillance tool needed in the “endgame” of schistosomiasis.

Author contributions: M.E.S., M.H., A.O., P.F.T., E.W., T.K.K., and B.J.V. designed research; M.E.S., M.H., H.C.K., A.O., H. Mejer, M.T.M., and H. Madsen performed research; M.H., P.F.T., and E.W. contributed new reagents/analytic tools; M.E.S., P.F.T., H. Madsen, A.-S.S., and B.J.V. analyzed data; M.E.S., M.H., A.O., A.-S.S., and B.J.V. wrote the paper with contributions from all other authors.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

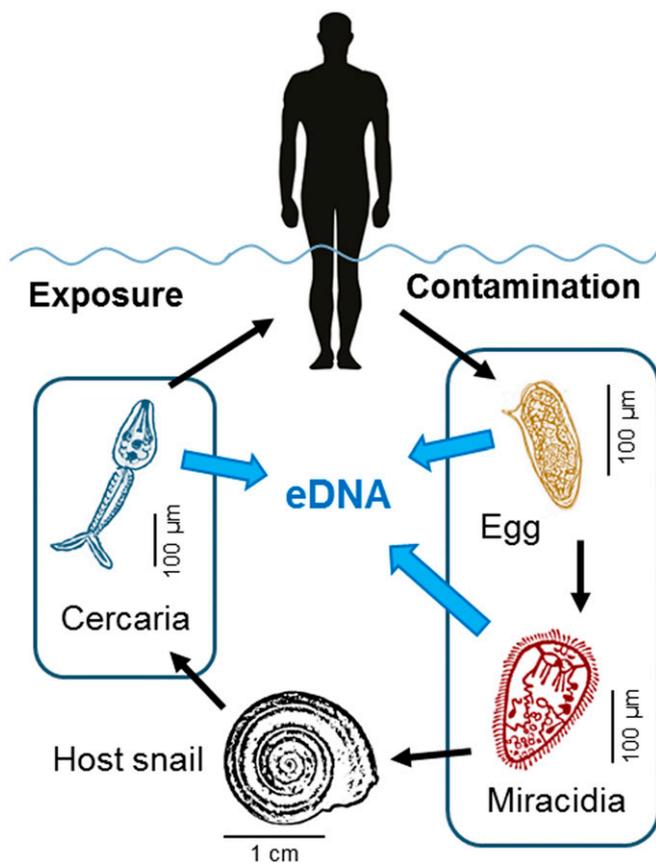
Published under the PNAS license.

<sup>1</sup>To whom correspondence should be addressed. Email: msen@sund.ku.dk.

<sup>2</sup>A.-S.S. and B.J.V. contributed equally to this work.

This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1815046116/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1815046116/-DCSupplemental).

Published online April 11, 2019.



**Fig. 1.** The life cycle of *S. mansoni*, illustrating main environmental aspects of transmission related to eDNA. Infected humans contaminate water sources via feces containing eggs, which hatch into miracidiae larvae infectious to snails of the genus *Biomphalaria*, that act as intermediate hosts. The presence of host snails is essential for further parasite development. After maturation inside the snail, thousands of cercariae are shed into the water, seeking a human host. Each day the emergence, death, and decay of the parasite larval stages, and possibly also eggs, contribute to the aquatic pool of eDNA.

percent of snail populations collected usually are found to be infected, even in areas with high human infection numbers (11–13). Even though introduction of DNA techniques for molecular detection of parasite infections in snails recently has revitalized traditional snail-monitoring methods (14, 15), extensive snail surveys are still needed to confirm parasite presence. Thus, more sensitive methods to detect schistosome larval stages directly in aquatic environments are still lacking (6, 16), especially for low-intensity infection areas.

To address this challenge, we set out to develop and test an alternative method to track parasite presence by detecting the traces of DNA, known as environmental DNA (eDNA) (17–19), left behind by the aquatic schistosome larval stages (miracidiae and/or cercariae; Fig. 1). Aquatic eDNA in general consists of nuclear or mitochondrial DNA released from organisms via feces, mucous, gametes, skin, hair, and carcasses and can be detected in cellular or extracellular form directly in the environment (20, 21). Our study was inspired by the recent and groundbreaking developments in eDNA research and its applications in the fields of conservation biology to detect and monitor rare, elusive, or invasive aquatic species (22, 23) and the hitherto-unexplored potential of eDNA methods in parasitology in general (24).

In the present study, we develop an eDNA-based method to detect the environmental stages of the parasite *Schistosoma*

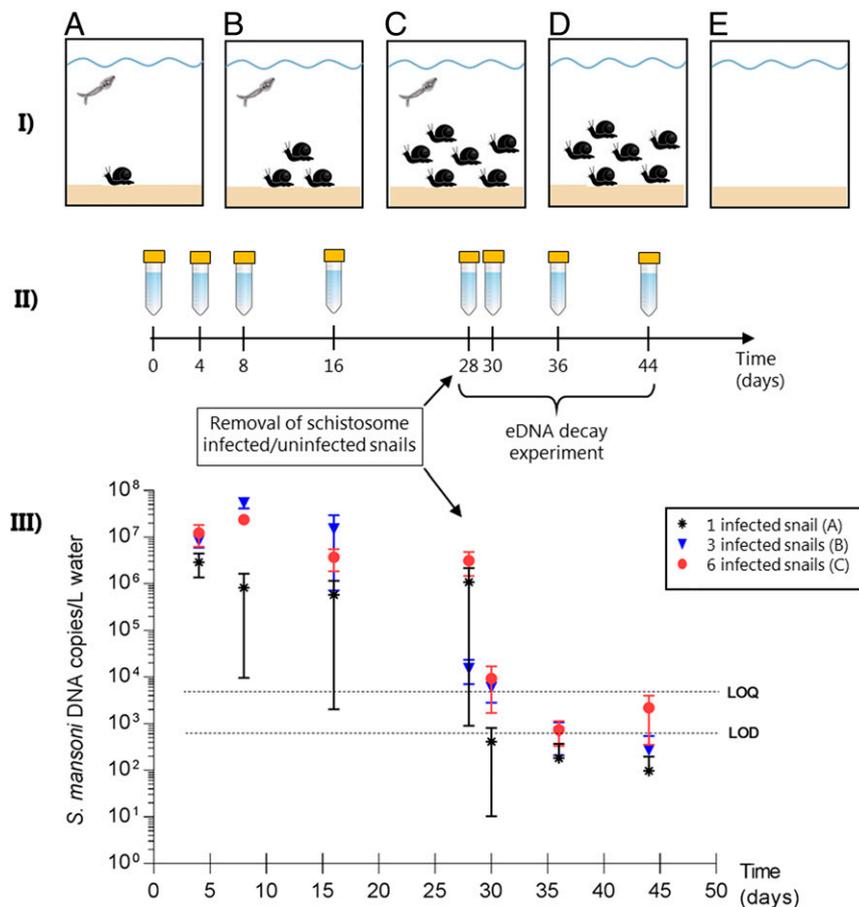
*mansoni*, causative agent of human intestinal schistosomiasis, in its aquatic environments. We design a species-specific TaqMan quantitative PCR (qPCR) assay and test this in laboratory-tank microcosm experiments to determine assay specificity and sensitivity, as well as schistosome eDNA decay. We then test the applicability and sensitivity of the approach at field sites in Kenya with known history of intestinal schistosomiasis transmission.

## Results

**Species-Specific qPCR Assay.** Primers and probe were designed to specifically target *S. mansoni* and then successfully validated to be species-specific in silico (database blast search; *SI Appendix, Fig. S1*), in vitro (on tissue-derived DNA extracts of target and nontarget schistosome species), and in situ (on DNA extracts from tank microcosm and field-collected water samples).

**Tank Experiment 1: Microcosm and Decay of *S. mansoni* eDNA.** To validate the species specificity and sensitivity of the qPCR assay, tank microcosms with varying densities of cercariae-shedding *Biomphalaria* host snails infected with *S. mansoni* (one, three, and six snails per tank) were sampled continuously over a 28-d period (Fig. 2 and *SI Appendix, Table S1*). Schistosome eDNA was detected in water samples at all three snail densities (tanks A–C in Fig. 2) already at the first sampling day and throughout the 28 d, reaching maximum concentration levels of  $2.9 \times 10^6$  (one-snail density on day 4),  $5.4 \times 10^7$  (three-snail density on day 8), and  $2.4 \times 10^7$  (six-snail density on day 8) *S. mansoni* DNA copies per liter of water (Fig. 2). However, a quantitative relationship was not observed between the number of infected snails and the number of schistosome DNA copies detected in the water. To determine schistosome eDNA decay, all snails were removed from the tanks on day 28, and water sampling was continued until day 44 (Fig. 2). The parasite eDNA concentrations declined rapidly from concentrations of  $1.1 \times 10^6$ ,  $1.5 \times 10^4$ , and  $3.2 \times 10^6$  DNA copies per liter of water for snail densities of one, three, and six, respectively, below level of quantification (LOQ; 10 DNA copies per qPCR reaction) and level of detection (LOD; one DNA copy per qPCR reaction) (*SI Appendix, Fig. S2*). Only the tank with three snails significantly fitted the simple exponential-decay model, and the estimated time for eDNA to degrade below LOQ and LOD (*SI Appendix, Fig. S3*) was estimated to be 3 and 8 d ( $P < 0.05$ ), respectively. However, it should be noted that it was not possible to fit decay models for the other two tank experiments (*SI Appendix, Fig. S3*) because the initial concentrations of eDNA were very high compared with other eDNA-degradation studies (22, 25) and dropped quickly below the level of detection (Fig. 2). For future eDNA decay experiments with parasites, we therefore recommend onset of sampling already on day 1 and sampling at smaller time intervals during the first days of the experiment. All water samples from the two control tanks (tanks D and E in Fig. 2) were negative, as well as all day-0 water samples from all tanks (tanks A–D in Fig. 2) before the addition of snails. All laboratory control samples were also negative, leaving no indication of contamination.

**Tank Experiment 2: Detection of True eDNA vs. Whole-Schistosome Cercariae.** To determine whether whole cercariae were captured during water sampling, a second tank experiment was performed. Sampling of water with the presence of whole cercariae was compared with sampling water with only true eDNA (with whole cercariae removed from water). Results clearly showed that the eDNA method was also able to trace true *S. mansoni* eDNA (Fig. 3). At all three cercariae densities (10, 100, and 1,000 cercariae per liter of water), the removal of cercariae lowered the average level of detected DNA copies considerably. Furthermore, a quantitative relationship was found between the density



**Fig. 2.** Overview of tank experiment 1 microcosms. (I) Experimental setup consisted of tanks ( $n = 3$ ) of schistosome-infected *Biomphalaria* snails at densities of one snail (tank A), three snails (tank B), and six snails (tank C) per tank (4 L) and two control tanks with six uninfected snails (tank D) and no snails (tank E). (II) Water for eDNA analyses was sampled on days 0 (before adding any snails), 4, 8, 16, 28 (all snails were removed), 30, 36, and 44. From day 28, eDNA decay was measured. (III) Results showing the concentration of *S. mansoni* eDNA (copies per liter of water;  $\pm$ SEM) on each sampling day with the schistosome-infected snail densities of one, three, and six per tank. The LOQ (10 DNA copies per qPCR reaction) and LOD (1 DNA copy per qPCR reaction) is shown to indicate the position of the data points in relation to these limits (SI Appendix, Fig. S1). The two control tanks (tanks D and E) revealed no amplification of *S. mansoni* eDNA and hence are not shown in the graph.

of cercariae and the amount of schistosome eDNA present in the water.

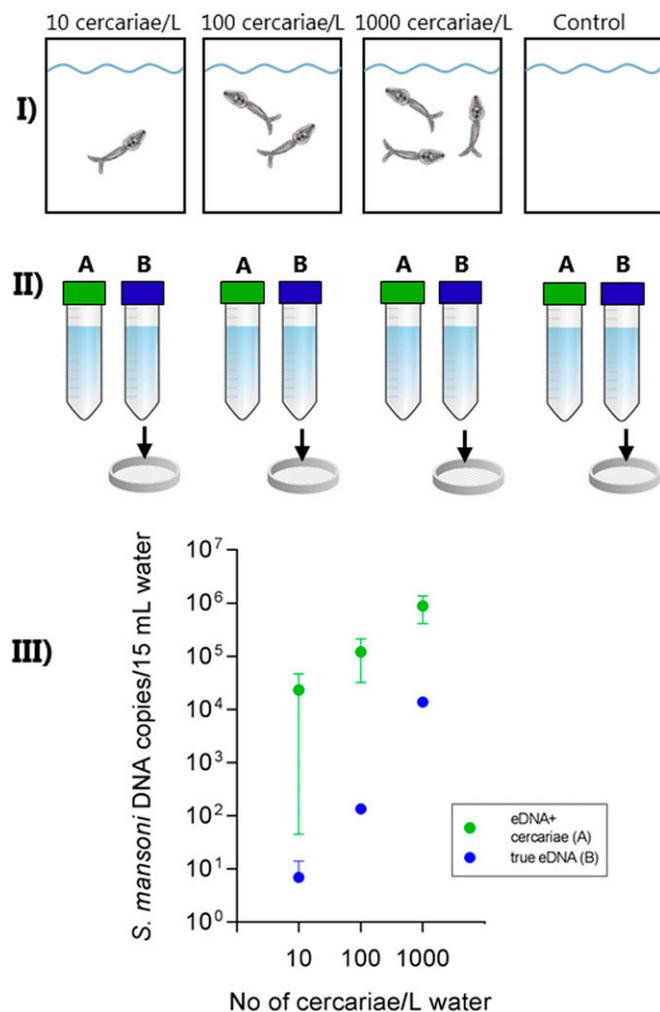
**Detection of *S. mansoni* at Field Sites Using eDNA and Snail Surveys.** With the eDNA method (qPCR on extracted water samples), *S. mansoni* was detected in water samples from four of five sites in central Kenya with known ongoing transmission (Fig. 4). By comparison, the conventional snail surveys (catching snails and shedding them by means of light stimulation, followed by PCR) failed to detect schistosome presence at two sites with known transmission (sites 1 and 2) (Fig. 4 and Table 1). At the two sites with no history of transmission (sites 6 and 7), no schistosome eDNA amplified in the water samples, and no host snails were found either. Overall, the two methods agreed in 71% of the cases (Fig. 4 and SI Appendix, Tables S2 and S3). The overall *S. mansoni* infection rate in the surveyed snail populations in Kenya measured by shedding was 0.4–2.2%.

Observed (naïve) detection probabilities at sites where *S. mansoni* was detected were higher for the eDNA method (0.33–0.67) than for conventional snail surveys (0.0004–0.02) (Table 1). The estimated number of water samples required to have a 95% probability to detect the presence of *S. mansoni* eDNA in at least one sample per site ranged from four to seven samples, whereas the conventional snail survey required between 148 and 747 snail

specimens of *Biomphalaria pfeifferi* from each site to achieve a similar level of detection (Table 1).

**Model-Based Estimates of eDNA Detection Probabilities in the Field.** To avoid overestimating the eDNA detection probability at field sites, which can arise from imperfect detection issues (26, 27), the eDNA data were analyzed by using a Bayesian multiscale occupancy model developed specifically for eDNA sampling designs (28). This approach allowed the estimation of eDNA occurrence and detection probabilities in relation to various biotic and abiotic factors that may influence detection probability (Table 2) at three hierarchical levels:  $\Psi$  (site level),  $\theta$  (water sample level), and  $\rho$  (qPCR replicate level).

The occupancy model with the best support [as measured by the posterior predictive loss criterion (PPLC) under squared-error loss and the widely applicable information criterion (WAIC)] included host snail presence as a covariate of eDNA occurrence probability at site level and conductivity as a covariate for eDNA detection probability in qPCR replicate level. A positive effect of snail presence on parasite eDNA site occupancy (Bayesian posterior median model estimate 1.85 [95% Bayesian credible interval (BCI) –0.24; 2.82]) and a negative influence of conductivity on eDNA detection probability in qPCR replicates [posterior median estimate of –0.38 (95% BCI –0.93; 0.15)] was observed (Fig. 5 and Table 3). However, the uncertainty of these parameters was high, leading to



**Fig. 3.** Overview of tank experiment 2. (I) Experimental tank setup with cercariae density of 10; 100; and 1,000 per liter of water, and a control tank with water only. (II) Two series (series A and B) of triplicate water samples for eDNA analyses were collected, and the B series of water samples was filtered to remove whole cercariae. (III) Results showing the concentration of *S. mansoni* eDNA (copies per 15 mL of water;  $\pm$ SEM) for each cercariae density. The control tank showed no amplification of *S. mansoni* eDNA and hence is not shown in the graph.

nonstatistically important effects (95% BCIs for both variables encompassed zero, as can be seen in *SI Appendix, Fig. S4*).

Based on the overall model, a total of seven water samples was required to achieve >95% detection probabilities of *S. mansoni* eDNA at water sample level ( $\theta = 0.35$ ) [as calculated by using the equation  $P = 1 - (1 - \theta)^n$  (27)]. By using the same approach, the model-based estimated number of qPCR replicates required to achieve detection probabilities >95% ranged from three to nine replicates between sites.

All parameter estimates (posterior medians and 95% BCIs) for the best-fitting eDNA occupancy model can be seen in Table 3 and *SI Appendix, Fig. S4*. All models and their ranking according to WAIC and PPLC can be seen in *SI Appendix, Table S4*.

**Comparison of Sampling Efforts and Associated Costs.** To investigate the potential cost-effectiveness of the eDNA approach, estimated sampling efforts and associated costs for a further improved eDNA tool and conventional snail sampling were compared for one site. Importantly, a main assumption was that the eDNA method for schistosome detection had been further

optimized, overcoming the challenges met by the present study. With this in mind, the estimated total effort spent on surveying one site using eDNA was similar to that of using traditional snail collection and shedding when collecting a low number of samples (i.e., three water samples and 148 snails) (Table 4), whereas with high sample numbers (i.e., seven water samples and 747 snails), the total efforts using eDNA were half of that of snail monitoring. The estimated cost for equipment needed for snail surveys and shedding (scoops, trays, and beakers) was generally very low, and the equipment could be reused several times, whereas enclosed filters and reagents for eDNA analysis cost ~165–385 US dollars per site.

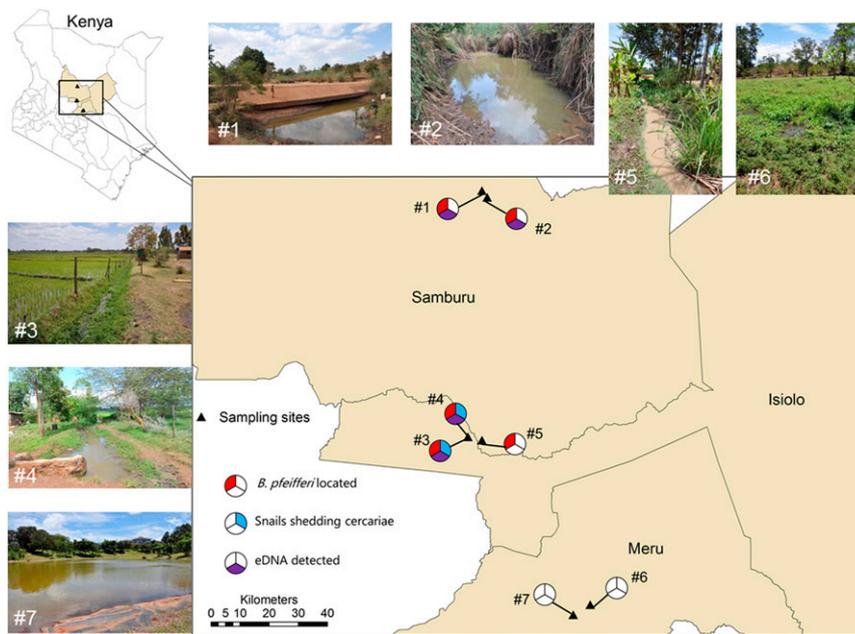
## Discussion

We here present a successful qPCR-based tool to detect eDNA from the snail-borne parasite *S. mansoni* directly in its freshwater habitat. The demonstrated high level of sensitivity of this eDNA approach to detect schistosome environmental larval stages will become increasingly important as environmental-transmission interruption becomes the measure of the true endpoint of schistosomiasis (5, 6, 29).

Earlier attempts to develop a molecular-detection method for environmental schistosome stages (30, 31) applied filtering of water using pore sizes appropriate for capturing cercariae, but too large to capture “true” eDNA. In the present study, by employing a state-of-the-art eDNA filtering process, we successfully demonstrate that the eDNA method does in fact also detect true schistosome eDNA together with whole-larval stages. This is essential, as these larval stages can be easily missed due to the highly spatial and temporal variation in snail and cercariae density under natural conditions. Moreover, the cercariae are short-lived, with a life expectancy of maximum 24 h, whereafter they die and degrade, and would thus be overlooked if water-sample filtering is done with too large pore sizes. True eDNA, on the other hand, persists for a longer time in the environment compared with cercariae.

Despite the obvious potential for applying eDNA for environmental surveillance of schistosomiasis, there are a number of limitations and challenges that should be addressed. First, for the time being, eDNA can only be used to determine the presence (or absence) of schistosomes at field locations, even though knowing the relative densities of parasite infective stages across the infection-risk landscape could also be very useful to guide schistosomiasis-control efforts. To determine schistosome parasite abundance, a quantitative relationship between the number of target organisms and eDNA molecules would be required, as demonstrated in other studies (e.g., refs. 22, 25, and 32). In the tank experiment 2, such a relationship between the number of cercariae and concentration of schistosome eDNA was indeed established (Fig. 3). However, even though the use of eDNA to quantify species abundances is currently a fast-growing field (e.g., refs. 33 and 34), some basic issues are still unresolved. Importantly, it remains to be explored how eDNA signals from organism abundance in natural water bodies can be differentiated from organism proximity to where the water samples are taken (35, 36).

Another pressing issue in eDNA studies in general is for how long DNA from an organism is traceable in aquatic environments after removal of the DNA source (37) and how different abiotic and biotic factors may affect eDNA detection in the field (38). This is also highly relevant for the applicability of the eDNA method for schistosome detection, since the parasite larval stages are relatively short-lived, as mentioned earlier. Our decay experiment (in the tank experiment 1) showed detectability of cercariae eDNA in the tank environment up to 8 d after the shedding event (Fig. 2). This decay is consistent with previous studies estimating the limit of aquatic eDNA detection to be between a couple days and up to several weeks after removal of the target organism (39–41). However, the decay of



**Fig. 4.** Overview of sampling sites in Kenya and detection results for the conventional snail-based survey (*B. pfeifferi* intermediate host snail location and shedding of cercariae) and the eDNA method (water sampling and qPCR analyses). Transmission of *S. mansoni* is known to be ongoing at sites 1–5, whereas sites 6 and 7 have no history of transmission.

schistosome eDNA at actual transmission sites, compared with controlled tank environments, would probably be faster than a week since the initial DNA concentration of the decay experiment was quite high in comparison with other eDNA decay studies (22, 25). Moreover, increased microbial activity, higher temperatures, and dispersal in natural waters could additionally accelerate the eDNA degradation (35, 42). Effects of abiotic and biotic factors on eDNA are shown to vary across species and ecosystems (38). According to our best-fitting eDNA occupancy model, we found that snail presence positively influenced parasite eDNA detection at site level, while conductivity had a negative effect on eDNA

detection at the qPCR level (although not important statistically). Furthermore, no factors seemed to influence the probability of detecting parasite eDNA at the water-sample level. These findings are in line with other eDNA detection studies examining the effect of covariates (Table 2), most recently Harper et al. (43).

Third, under field conditions, it is not possible to determine if the schistosome DNA source originates from cercariae (the human-infective stage) or miracidiae (the snail-infective stage). This means that the eDNA method cannot at this stage separate detection of contamination (input of miracidiae from infected humans) from exposure potential (snail output of cercariae infective to humans)

**Table 1.** Survey metrics and observed (naïve) detection probabilities of *S. mansoni* using either snail shedding or eDNA monitoring across seven sites in Kenya

Survey metrics	Survey site number						
	1	2	3	4	5	6	7
Positive samples* (detection probability)							
Snail shedding <sup>†</sup>	0/11 (0.00)	0/2 (0.00)	1/240 (0.004)	1/45 (0.02)	0/73 (0.00)	0/0 (0.00)	0/0 (0.00)
eDNA in water samples <sup>‡</sup>	1/3 (0.33)	2/3 (0.67)	1/3 (0.33)	1/3 (0.33)	0/3 (0.00)	0/3 (0.00)	0/3 (0.00)
qPCR	3/9 (0.33)	2/6 (0.33)	6/9 (0.67)	1/9 (0.11)	0/9 (0.00)	0/6 (0.00)	0/6 (0.00)
	0/9 (0.00)	3/6 (0.50)	0/9 (0.00)	0/9 (0.00)	0/9 (0.00)	0/6 (0.00)	0/6 (0.00)
	0/9 (0.00)	0/6 (0.00)	0/9 (0.00)	0/9 (0.00)	0/9 (0.00)	0/6 (0.00)	0/6 (0.00)
Estimated number of samples, <i>n</i> , for $P > 0.95^{\S}$							
Snails	—	—	747	148	—	—	—
Water samples	7	3	7	7	—	—	—
qPCR replicates	7	7	3	26	—	—	—
	—	4	—	—	—	—	—
	—	—	—	—	—	—	—

\*Values are the number of positive snails or water samples out of the total number of collected samples.

<sup>†</sup>Number of snails shedding *S. mansoni* cercariae out of the total number of collected snails at each site.

<sup>‡</sup>A water sample is designated positive for *S. mansoni* presence if one or more qPCR replicates amplified *S. mansoni* DNA.

<sup>§</sup>The number of samples (*n*) (snails or water samples) required for the *S. mansoni* site detection probability (*P*) to exceed 0.95 is calculated by using the equation  $P = 1 - (1 - p)^n$ , where *p* is the observed (naïve) detection probabilities at a given site. —, not applicable.

**Table 2. Measured biotic and abiotic factors that potentially influence field-site eDNA availability and detection probability and included as covariates in the eDNA occupancy modeling analysis**

Model covariates	Hypothesized effects	Source
<i>Biomphalaria</i> snail presence/absence or density	Presence of snails at a site could be indicative of the presence of parasite eDNA.	Present study
Presence of snails shedding cercariae	Presence of shedding parasites could increase the parasite eDNA concentration in the water and thus improve detection probability.	Present study
Salinity	Higher salinity can result in higher availability probability of eDNA due to increased DNA stability.	Ref. 66
Temperature	Affects the physical and metabolic activity of organisms; faster degradation at higher temperatures can lead to lower availability probability of eDNA.	Refs. 67 and 68
Conductivity	Relates to total dissolved solids, which can impair eDNA detection due to release of inhibitory substances and their capacity to bind DNA.	Refs. 38, 43, and 50

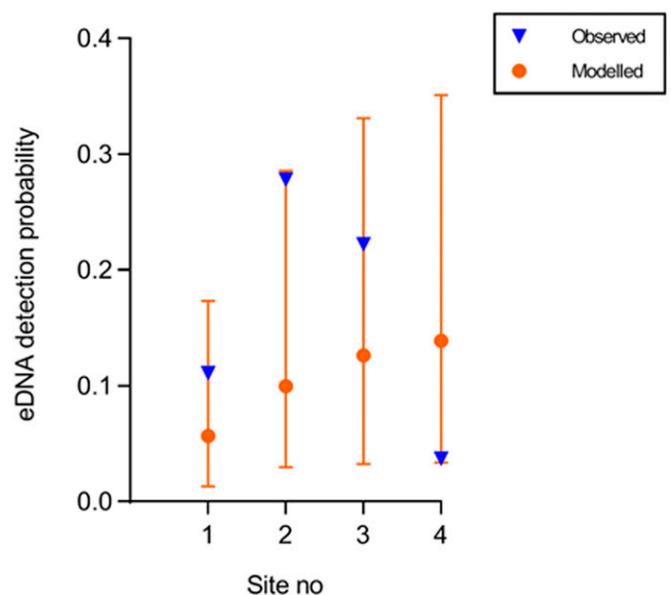
(Fig. 1; ref. 6). However, from an elimination point of view, detection of cercariae as well as miracidiae is important, since detection of miracidiae would imply that the parasite is present and is potentially completing its life cycle via reservoir hosts [rodents and other wild animals (44)]. Often, this would indeed suggest that transmission has not been interrupted. Furthermore, we cannot be sure whether the schistosome eDNA arises from living or dead parasite larval stages, which also could pose a challenge when assessing real-time transmission (45). However, this concern is somewhat unjustified, since the short timespan for schistosome eDNA degradation is at maximum a week, and thus eDNA detection of schistosome presence would indeed represent ongoing potential transmission.

A mechanical challenge met during the field testing was filtering of turbid water using pore sizes small enough to capture true eDNA (0.22  $\mu\text{m}$ ). Usage of several filter units per water sample (*SI Appendix, Table S3*) was necessary due to clogging of filters, even though prefiltering (with pore size 350  $\mu\text{m}$ ) was used to remove larger particles (46). Application of other eDNA-capture methods—i.e., other filter types—has not proved to be as efficient as the enclosed filters used in the present field study (47). Additionally, using the enclosed filter units in the field reduces the risk of contamination, since the filters are never openly exposed. Future studies should focus on how to filter the required volume of water with varying turbidity and simultaneously capture of small DNA fragments to keep the number of enclosed filter units at a minimum and reduce subsequent laboratory work time.

To be applicable for larger-scale schistosomiasis surveillance and control programs, the eDNA method would naturally need further refinement. Thus, testing and validation of the method performance under a variety of field conditions and habitats in areas with well-known histories of schistosomiasis transmission would be valuable. Evidence from other eDNA field-based studies on various organisms demonstrates that eDNA improves detection of rare or cryptic species (48, 49). Schistosomes are likewise difficult to detect due to their very small size. Transportation of eDNA downstream with flowing water has been shown to take place (50). This would also need to be accounted for in certain habitat types (i.e., irrigation schemes), since the point of schistosome contamination could be further upstream. Outstanding questions relate to if and how different habitat types (flowing vs. stagnant waters) or seasonal variation in snail populations—and hence also schistosome populations—influence schistosome eDNA presence, concentration, and detection probability, especially since seasonality in organism abundances is found to be reflected in the aquatic eDNA concentrations (51). A next critical step would then be to develop a panel of sampling guidelines and strategies for eDNA application according to season, habitat type, and the type of transmission setting (52), which probably influence the

recommended numbers of water samples, the temporal sampling frequency, and the ideal spatial sampling at each habitat type.

Regardless of these challenges, the relatively rapid field-collection procedure and simple field equipment combined with a high sensitivity means that eDNA sampling could be widely applicable for broad-scale environmental surveillance of schistosomiasis, especially in low-transmission areas. The feasibility of eDNA in this context will, however, depend critically on the associated costs and required efforts of the method. The estimated total sampling efforts for a further optimized eDNA sampling and filtering procedure indicates that man-hours spent per field site are similar or reduced compared with the time spent for conventional snail surveys, depending on number of samples (Table 4), which could make a difference in terms of salary expenses. Numerous water samples collected for eDNA analyses would increase the total expenses of the method, as opposed to the snail collection and shedding, where materials are very cheap and reusable (Table 4). However, eDNA costs will most likely decrease over time, while the costs of snail surveys will remain unchanged, since salary represents the main expense (53).



**Fig. 5.** The overall eDNA occupancy model-derived probability of having a positive qPCR test at sites where eDNA was detected (sites 1–4), compared with the observed frequencies of positive qPCRs at the same sites. The model-derived, unconditional probability (median and 95% BCIs) of having a positive qPCR test at a site is calculated as the joint probability ( $\Psi \times \theta \times \rho$ ) by multiplying 500 samples drawn from the posterior distributions for each parameter.

**Table 3. Bayesian posterior estimates of *S. mansoni* eDNA occurrence probability at Kenyan field site ( $\psi$ ), schistosome eDNA detection probability in a water sample ( $\theta$ ), and schistosome eDNA detection probability in a qPCR replicate ( $\rho$ )**

Site	Snail presence, Y/N	Conductivity, mS	Median (95% BCI)		
			$\Psi$	$\theta$	$\rho$
1	Y	1.34	0.74 (0.29; 0.97)	0.35 (0.12; 0.70)	0.29 (0.08; 0.59)
2	Y	0.81	0.74 (0.29; 0.97)	0.35 (0.12; 0.70)	0.46 (0.28; 0.64)
3	Y	0.33	0.74 (0.29; 0.97)	0.35 (0.12; 0.70)	0.62 (0.34; 0.84)
4	Y	0.14	0.74 (0.29; 0.97)	0.35 (0.12; 0.70)	0.67 (0.33; 0.91)
5	Y	0.06	0.74 (0.29; 0.97)	0.35 (0.12; 0.70)	0.70 (0.33; 0.93)
6	N	0.53	0.16 (0.01–0.94)	0.35 (0.12; 0.70)	0.55 (0.33; 0.75)
7	N	0.17	0.16 (0.01–0.94)	0.35 (0.12; 0.70)	0.67 (0.33; 0.90)

Parameter estimates (posterior medians and 95% BCIs) are given for each parameter based on for the best-fitting eDNA occupancy model [ $\Psi$ (snailpres),  $\theta$ (.), or  $\rho$ (cond)]. N, no; Y, yes.

Applying PCR to detect parasites in snails would increase the snail-collection method sensitivity (14), but also increase total expenses, and prolonged shedding periods (54) would be highly impractical in a control program context. In general, the eDNA method is more versatile in the sense that it only depends on the presence of water, whereas snail collection also depends on the timing of collection (yearly seasonality) and shedding (daily peak in cercarial production) (55). Depending on the number of samples, the overall cost-effectiveness of the eDNA method is either comparable to snail shedding (if only a few samples are required) or higher (if many samples are required). This is line with other eDNA studies that

have compared eDNA with conventional monitoring methods and concluded that eDNA can reduce total survey costs (48, 56).

In the near future, to be able to proceed toward the end goal of schistosomiasis elimination, the ongoing transition from infection control to transmission control of schistosomiasis is at a critical point, where general guidelines are seriously needed (6). Recently, WHO published new guidelines for field application of chemical-based snail control (57), but no standard guidelines exist on how to carry out sensitive environmental surveillance. Naturally, eDNA methods cannot stand alone, but in areas with ongoing integrated control of MDA and snail control, the eDNA

**Table 4. Estimated efforts and costs for materials for sampling and analyses using the eDNA method and the conventional snail-based method (snail collection and shedding) to detect schistosomes**

Sampling method	eDNA		Snail survey	
	3 water samples per site	7 water samples per site	148 host snails per site	747 host snails per site
<b>Efforts</b>				
Collection of water samples and filtration*/collection of host snails <sup>†</sup> , man-hours	1.8	4.1	0.8	2.8
Labwork: extraction to qPCR <sup>‡</sup> /snail shedding <sup>§</sup> , man-hours	1.8	2.9	2.4	12.0
Total effort (per site), man-hours	3.6	7.0	3.2	14.8
<b>Materials</b>				
Collection of water samples and filtration <sup>¶</sup> , USD	45	105	0	0
DNA extraction and qPCR <sup>#</sup> , USD	120	280	0	0
Total cost (per site), USD	165	385	0	0

Estimated efforts are in man-hours per site, and costs for materials are in USD per site. Sample number estimations are made based on the lowest and highest number of samples (water samples and snails, respectively) required per site to reach a 95% detection probability (from Table 1). Here, we assume that the required number of snails (148 and 747) is sampled at one site by scooping for 20 min, which is somewhat of an underestimation of man-hours, since exploratory sampling at several sites before locating snail populations is often the reality. A schistosome infection rate of 2% is assumed for the snail populations. The analysis time for the qPCR machine (2 h per 96 samples) and shedding time for snails (4-h light stimuli) has not been included, since the man-hours can be used elsewhere during that time.

\*For collection and filtration, 35 min was spent per water sample.

<sup>†</sup>Collection of host snails includes 20 min of scooping (regardless of number of snails found), and sorting of snails is estimated to be 30 min per 150 snails.

<sup>‡</sup>eDNA laboratory work: DNA extraction (10 min per sample for preparing lysis and 30 min for extracting plus 2 min extra per sample), setting up qPCR (in seven replicates, takes 20 min per sample plus 2 min extra per sample), and preparation time for sending a subset of samples for sequencing (10 min per sample plus 2 min extra per sample) has been included to verify target species.

<sup>§</sup>Snail shedding includes mass shedding of snails (10 snails per beaker and 3 min per beaker), checking for cercariae (2 min per beaker), individual shedding of infected snails (assumed to be 2% of total collected host snails; thus, 3 and 15 mass-shedding beakers set up for individual shedding, 3 min per beaker), and checking individual beakers for cercariae (2 min per beaker).

<sup>¶</sup>Materials for water collection and filtration are estimated to be 15 USD per water sample.

<sup>#</sup>Reagents for DNA extraction, qPCR, and sequencing are estimated to be 40 USD per water sample.

method could provide an additional highly accurate means to evaluate control efforts (45). For instance, the eDNA method could be used for closely monitoring locations declared free of transmission, but where there is a risk of reestablishment of transmission—e.g., due to the presence of nonhuman reservoir hosts—where infection might reside undetected by conventional methods. Additionally, early detection of emerging schistosomiasis outside the normally considered endemic range areas using eDNA could be useful to help prevent the disease from spreading. This could, for instance, be highly relevant in situations where schistosomiasis is moving into new territories, as seen recently in Corsica (France) in Europe due to substantial human migration from endemic transmission areas (58, 59), or due to climate change making new areas suitable for the establishment of both intermediate host snail species and the parasite (60).

Finally, the possibility for eDNA methods to include detection of additional species from the same water samples—i.e., schistosome host snail species (*Biomphalaria* sp., *Bulinus* sp., or *Oncomelania hupensis*) or other schistosome species (*Schistosoma haematobium* or *S. japonicum*)—could make the method a true “game-changer” in schistosomiasis environmental surveillance and control. Alternatively, application of the eDNA metabarcoding approach (46) detecting overall species richness in natural environments using high-throughput sequencing of eDNA could be feasible (33). Especially since snail control is now again emphasized in the plans to eliminate schistosomiasis (45), eDNA detection of schistosome host snail species offers a promising supplement to the conventional snail surveys to help pinpoint “transmission hotspots” (29). The relative ease with which water samples can be collected means that larger geographical areas could be sampled—i.e., through citizen science programs (56). Thus, eDNA could potentially boost the currently scarce amount of empirical data on host snail and parasite spatiotemporal distributions. These data would allow improved species distribution and risk models, and hence more detailed “real-time” risk maps of schistosomiasis transmission in both emerging and endemic countries, as well as for predicting future risk scenarios under climate change (61).

## Materials and Methods

**Design and Validation of Species-Specific Primers.** Species-specific primers and probe targeting a 86-bp-long sequence in the mitochondrial gene cytochrome oxidase I (COI) of *S. mansoni* (Schiman\_COIF: 5'-ATTTACGGTTGGTGTGCA-3'; Schiman\_COIR: 5'-GAGCAACAACAA ACCAAGTATCA; Schiman\_COLprobe: Fam-GGGGTGGCTTTATCTGCATCTGC-BHQ-1-3') were designed for this study by visual comparison with aligned sequences of *S. mansoni* and other closely related nontarget schistosome species occurring in East Africa obtained from NCBI GenBank (SI Appendix, Fig. S1). Primer and probe sequence motifs were selected with the least theoretical risk of cross-species amplification with nontarget species and validated in silico. The primer/probe species-specificity was validated in vitro by real-time qPCR of genomic DNA tissue extracts from the target species *S. mansoni* and tested negative for the closely related nontarget species *Schistosoma rhodaini*, *S. hematobium*, and *Schistosoma bovis*.

**Tank Experiment 1: Microcosms and eDNA Decay.** To assess the efficiency and reliability of this proposed eDNA tool, the primer specificity and sensitivity was first validated in situ in laboratory-based tank experiments (microcosms) housing different densities of intermediate host snails, *Biomphalaria glabrata*, infected with *S. mansoni* (see Fig. 2 for experimental setup). Water samples were collected (for ethanol precipitation) before introduction of infected snails (day 0) and at days 4, 8, 16, and 28. Hereafter, snails were removed, and sampling of water was continued on days 30, 36, and 44 to examine degradation of schistosome eDNA. Water samples were analyzed by using qPCR to quantify DNA amounts and sequenced to confirm *S. mansoni* eDNA.

**Tank Experiment 2: Detection of True eDNA vs. Whole Cercariae.** To clarify the possible effect of capturing whole cercariae vs. true eDNA when sampling water for *S. mansoni* detection, the tank experiment 2 with different cercariae densities (10, 100, and 1,000 cercariae per liter of water) was set up (Fig. 3). Two series (A and B) of triplicate water samples were taken from each cercariae density, and series B samples were filtered (pore size 12 μm) to remove whole cercariae from the water sample, after which all of the

samples were precipitated. Quantification of *S. mansoni* DNA copies was determined by using qPCR.

**Comparison of eDNA Method and Snail Survey in Field Sites in Kenya.** The eDNA method was validated in September 2015 in central Kenya at a total of seven field sites with known ongoing transmission or with no history of transmission (Fig. 4, Table 1, and SI Appendix, Table S2). The timing of snail collection was planned to take place in the second of two transmission seasons occurring in the particular area and was based on prior local knowledge (62, 63). At each site, a water body with human activity was selected, and water samples for eDNA analyses were taken before the conventional snail-based survey. For eDNA analyses, triplicate water samples of 1 L were taken from a pond (sites 1, 2, 6, and 7) or a stream (sites 3, 4, and 5). A 1-L container with a prefilter (pore size 350 μm) attached to remove large particles was submerged just below the water surface and filled. The water samples were taken from the water-body edge by reaching out, wearing long sterile gloves and without stepping into the water. All field equipment was sterilized in 10% bleach solution and thoroughly dried between sites. Water samples were placed on ice in a dark container immediately after collection until being filtered with enclosed Sterivex-filters (0.22 μm) by using a vacuum pump. Enclosed filters containing eDNA were preserved with RNAlater and kept at −20 °C until DNA extraction, following Spens et al. (47). It should be noted that, for field application, storing samples at −20 °C is not a requirement, as RNAlater protects the sample. Amplification of *S. mansoni* DNA was done by using qPCR.

Conventional snail surveys were performed at each site by catching snails using a scoop for 20 min covering the selected sampling site (63). All specimens of *B. pfeifferi* (the intermediate host snail species in central Kenya) were identified based on shell morphology (64) and set up for shedding of cercariae in small beakers placed in the light (sun or artificial) for at least 4 h as light stimuli induce shedding (10, 57). When a large number of snails was scooped, the snails were set up for mass shedding of 10 snails in each beaker. All beakers were then visually inspected under a microscope, and, if the fork-tailed schistosome cercariae were detected, the 10 snails were singled out in separate beakers to identify the exact snail shedding cercariae. All of the host snails were preserved in ethanol 96%. The *S. mansoni* infection of the positive host snails was confirmed by using qPCR.

**eDNA Decay.** An exponential decay model was fitted to the qPCR data from day 28 (set to  $t = 0$ , as snails were removed) up to day 44 from the microcosm experiment, as this is the relationship most evident for molecular decay as shown by Schnell et al. (65). The decay model is the following:

$$\frac{dN}{dt} = -\beta N.$$

Solving this gives:

$$N(t) = N_0 e^{-\beta t}.$$

$N(t)$  is the DNA concentration at time  $t$ . The two parameters  $N_0$  (initial DNA concentration at  $t = 0$ ) and  $\beta$  (decay constant) were estimated by the nls function in R (Version 3.4.4), resulting in the values  $N_0 = 15.19$  DNA copies per liter of water and  $\beta = 0.46 \text{ d}^{-1}$  for *S. mansoni* in the three-snail aquaria (tank B in Fig. 2). These parameters were used to calculate after how many days ( $t$ ) DNA levels would reach beyond LOQ and LOD.

**eDNA Occupancy Modeling.** The R package eDNAoccupancy (Version 0.2.0) (28) was used to fit Bayesian, multiscale occupancy models to estimate schistosome eDNA occurrence and detection probabilities. This approach allowed us to estimate parasite eDNA occurrence and detection probabilities at several hierarchical levels, while also taking the potential effects of environmental covariates into account. The nested survey designs in the present study are common for many eDNA surveys (26, 28, 43) and included the following: (i) the site occupancy probability ( $\psi_i$ ), defined as the probability of schistosome occurrence at site  $i$ ; (ii) the availability probability ( $\theta_{ij}$ ), defined as the probability of schistosome eDNA being available for detection in water sample  $j$  given that it is present at site  $i$ ; and (iii) the conditional probability of schistosome detection ( $p_{ijk}$ ), defined as the probability of schistosome eDNA detection in qPCR replicate  $k$  given that it is present in the water sample  $j$  and site  $i$ .

Several biotic and abiotic factors may potentially affect eDNA detection, persistence, and degradation, according to the eDNA literature (Table 2), and, therefore, we constructed several models to compare the relative importance of these factors. Specifically, we hypothesized that sites with a presence of intermediate host snail species, observed shedding, or high density of snails would have a higher site eDNA occupancy probability ( $\psi$ ),

whereas detection probability in water samples ( $\theta$ ) was hypothesized to decrease with increasing salinity, temperature, and conductivity. Finally, higher salinity and conductivity were hypothesized to result in inhibition and therefore decrease detection probability at the qPCR level ( $\rho$ ) (see Table 2 for summary of potential effects of biotic and abiotic factors). More details on model formulation can be found in *SI Appendix, section S1*.

In total, 64 models were constructed, which included snail-related covariates at site level ( $\Psi$ ) and a combination of temperature, conductivity, and salinity at the water-sample level ( $\theta$ ) and conductivity and salinity at the qPCR replicate level ( $\rho$ ). All models were fitted by running a Markov chain Monte Carlo (MCMC) algorithm for 11,000 iterations and retaining the last 10,000 for estimating posterior summaries.

Models were ranked (*SI Appendix, Table S4*) according to PPLC under squared-error loss and the WAIC. Models with lower values of these criteria were favored. Exploratory analysis showed that attempts to fit models with multiple covariates for a single parameter led to overfitting and substantially higher PPLC and WAIC estimates. Thus, according to the principle of parsimony, we favored models with fewer predictors. The best model was then fitted by running the MCMC algorithm for 100,000 iterations and retaining the last 50,000 iterations for posterior value estimation. Convergence of the Markov chain used to compute the model estimates was assessed through trace plots of the parameters (28). Finally, the equations  $1 - (1 - \hat{\theta})^n = 0.95$  and  $1 - (1 - \hat{\rho})^n = 0.95$  (26, 27) were used to determine the number of water samples and qPCR replicates required for detection probabilities to exceed 0.95.

**Comparison of Sampling Efforts and Costs.** Assuming that the eDNA method for schistosome detection had been further optimized, overcoming the challenges met by the present study, the potential cost-effectiveness of the eDNA approach per site was explored. For each of the two monitoring methods, the total effort (measured in man-hours) was estimated based on the lowest and highest number of samples required per site for 95% detection probability (Table 1). For the eDNA method, this includes sample collection as well as laboratory work, and for the conventional snail survey,

collection and shedding of snails and visual inspection of cercariae were included.

The cost of materials and reagents for eDNA analysis was estimated, including the cost of extraction, qPCR reagents, and commercial Sanger sequencing. Availability of a qPCR machine and other laboratory equipment was assumed, and the cost of various plastics, such as pipette tips and tubes, was not included in calculations. Likewise, the cost of snail sampling and shedding gear, such as metal mesh paddle scoops, plastics, and microscopes, was not included in the cost of snail surveys. Costs for travel, subsistence, and salaries were not included in these estimates, as they can vary substantially from country to country.

**Ethical Statements.** Infection of snails for the microcosm experiment was done with *S. mansoni* parasite material recovered from infected mouse livers delivered by Michael J. Doenhof, University of Nottingham, Nottingham, United Kingdom. During field sampling, collected host snails were not returned to the sites regardless of infection status due to the risk of prepatent infections in the snails.

**ACKNOWLEDGMENTS.** We thank the technical staff at Kimbimbi Health Centre in Mwea and Lise-Lotte Christiansen and Rolf Difborg from the University of Copenhagen for their immense help during field sampling; Susanne Kronborg for helping with experimental snail infections and the tank experiments; Tina Brand, Eva Egelyng Sigsgaard, and Steen Wilhelm Knudsen at the Centre for GeoGenetics for laboratory assistance; Penelope Vounatsou from the Swiss Tropical and Public Health Institute for stimulating discussion about eDNA occupancy modeling; and the National History Museum, London, for providing *S. rhodaini* worm material. E.W. thanks St. John's College, Cambridge, for inspiring scientific discussions. This work was supported by the Augustinus Foundation and Knud Højgaard's Foundation. E.W. was supported by the Danish National Research Foundation, the Lundbeck Foundation, and KU2016. A.-S.S. thanks the Knud Højgaard's Foundation for supporting the Research Platform for Disease Ecology, Climate and Health, and is grateful to the Danish National Research Foundation for supporting the Center for Macroecology, Evolution and Climate (Grant DNRF96).

- McManus DP, et al. (2018) Schistosomiasis. *Nat Rev Dis Primers* 4:13.
- Hotez PJ, et al. (2014) The global burden of disease study 2010: Interpretation and implications for the neglected tropical diseases. *PLoS Negl Trop Dis* 8:e2865.
- Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J (2006) Schistosomiasis and water resources development: Systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis* 6:411–425.
- World Health Organization (2013) Schistosomiasis: Progress report 2001–2011 and strategic plan 2012–2020 (WHO, Geneva).
- Bergquist R, Zhou X-N, Rollinson D, Reinhard-Rupp J, Klohe K (2017) Elimination of schistosomiasis: The tools required. *Infect Dis Poverty* 6:158.
- Stothard JR, et al. (2017) Towards interruption of schistosomiasis transmission in sub-Saharan Africa: Developing an appropriate environmental surveillance framework to guide and to support 'end game' interventions. *Infect Dis Poverty* 6:10.
- World Health Assembly (2012) Elimination of schistosomiasis. *Sixty-Fifth World Health Assembly: Resolutions and Decisions Annexes*, Report WHA65/2012/REC/1 (WHO, Geneva), 247–249.
- Spear RC, et al. (2011) The challenge of effective surveillance in moving from low transmission to elimination of schistosomiasis in China. *Int J Parasitol* 41:1243–1247.
- Wang L-D, et al. (2009) A strategy to control transmission of *Schistosoma japonicum* in China. *N Engl J Med* 360:121–128.
- Madsen H (1985) *Ecology and Control of African Freshwater Pulmonate Snails. Part I and II* (Danish Bilharziasis Laboratory, Charlottenlund, Denmark).
- Angelo T, et al. (2014) Population abundance and disease transmission potential of snail intermediate hosts of human schistosomiasis in fishing communities of Mwanza region, north-western, Tanzania. *Int J Sci Res* 3:1230–1236.
- Standley CJ, et al. (2010) Epidemiology and control of intestinal schistosomiasis on the Sesse Islands, Uganda: Integrating malacology and parasitology to tailor local treatment recommendations. *Parasit Vectors* 3:64.
- Opisa S, Odiere MR, Jura WGZO, Karanja DMS, Mwinzi PNM (2011) Malacological survey and geographical distribution of vector snails for schistosomiasis within informal settlements of Kisumu city, western Kenya. *Parasit Vectors* 4:226.
- Hamburger J, et al. (1998) A polymerase chain reaction assay for detecting snails infected with bilharzia parasites (*Schistosoma mansoni*) from very early prepatency. *Am J Trop Med Hyg* 59:872–876.
- Hanelt B, Adema CM, Mansour MH, Loker ES (1997) Detection of *Schistosoma mansoni* in *Biomphalaria* using nested PCR. *J Parasitol* 83:387–394.
- Rollinson D, et al. (2013) Time to set the agenda for schistosomiasis elimination. *Acta Trop* 128:423–440.
- Willerslev E, et al. (2003) Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* 300:791–795.
- Bohmann K, et al. (2014) Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol Evol* 29:358–367.
- Thomsen PF, Willerslev E (2015) Environmental DNA—An emerging tool in conservation for monitoring past and present biodiversity. *Biol Conserv* 183:4–18.
- Turner CR, et al. (2014) Particle size distribution and optimal capture of aqueous microbial eDNA. *Methods Ecol Evol* 5:676–684.
- Nielsen KM, Johnsen PJ, Bensasson D, Daffonchio D (2007) Release and persistence of extracellular DNA in the environment. *Environ Biosafety Res* 6:37–53.
- Thomsen PF, et al. (2012) Monitoring endangered freshwater biodiversity using environmental DNA. *Mol Ecol* 21:2565–2573.
- Ficetola GF, Miaud C, Pompanon F, Taberlet P (2008) Species detection using environmental DNA from water samples. *Biol Lett* 4:423–425.
- Bass D, Stentiford GD, Littlewood DTJ, Hartikainen H (2015) Diverse applications of environmental DNA methods in parasitology. *Trends Parasitol* 31:499–513.
- Harper KJ, Anucha NP, Turnbull JF, Bean CW, Leaver MJ (2018) Searching for a signal: Environmental DNA (eDNA) for the detection of invasive signal crayfish, *Pacifastacus leniusculus* (Dana, 1852). *Manag Biol Invasions* 9:137–148.
- Schmidt BR, Kéry M, Urnsbacher S, Hyman OJ, Collins JP (2013) Site occupancy models in the analysis of environmental DNA presence/absence surveys: A case study of an emerging amphibian pathogen. *Methods Ecol Evol* 4:646–653.
- Smart AS, Tingley R, Weeks AR, van Rooyen AR, McCarthy MA (2015) Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecol Appl* 25:1944–1952.
- Dorazio RM, Erickson RA (2018) ednaoccupancy: An R package for multiscale occupancy modelling of environmental DNA data. *Mol Ecol Resour* 18:368–380.
- Kincaid-Smith J, Rey O, Toulza E, Berry A, Boissier J (2017) Emerging schistosomiasis in Europe: A need to quantify the risks. *Trends Parasitol* 33:600–609.
- Hung YW, Remais J (2008) Quantitative detection of *Schistosoma japonicum* cercariae in water by real-time PCR. *PLoS Negl Trop Dis* 2:e337.
- Worrell C, et al. (2011) Field detection of *Schistosoma japonicum* cercariae in environmental water samples by quantitative PCR. *Appl Environ Microbiol* 77:2192–2195.
- Pilliod DS, Goldberg CS, Arkle RS, Waits LP (2013) Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Can J Fish Aquat Sci* 70:1123–1130.
- Deiner K, et al. (2017) Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Mol Ecol* 26:5872–5895.
- Lacoursière-Roussel A, Côté G, Leclerc V, Bernatchez L (2016) Quantifying relative fish abundance with eDNA: A promising tool for fisheries management. *J Appl Ecol* 53: 1148–1157.
- Barnes MA, Turner CR (2016) The ecology of environmental DNA and implications for conservation genetics. *Conserv Genet* 17:1–17.
- Carraro L, Hartikainen H, Jokela J, Bertuzzo E, Rinaldo A (2018) Estimating species distribution and abundance in river networks using environmental DNA. *Proc Natl Acad Sci USA* 115:11724–11729.
- Dejean T, et al. (2011) Persistence of environmental DNA in freshwater ecosystems. *PLoS One* 6:e23398.
- Barnes MA, et al. (2014) Environmental conditions influence eDNA persistence in aquatic systems. *Environ Sci Technol* 48:1819–1827.

39. Lance RF, et al. (2017) Experimental observations on the decay of environmental DNA from bighead and silver carps. *Manag Biol Invasions* 8:343–359.
40. Bylemans J, Furlan EM, Gleeson DM, Hardy CM, Duncan RP (2018) Does size matter? An experimental evaluation of the relative abundance and decay rates of aquatic environmental DNA. *Environ Sci Technol* 52:6408–6416.
41. Seymour M, et al. (2018) Acidity promotes degradation of multi-species environmental DNA in lotic mesocosms. *Commun Biol* 1:4.
42. Pilliod DS, Goldberg CS, Arkle RS, Waits LP (2014) Factors influencing detection of eDNA from a stream-dwelling amphibian. *Mol Ecol Resour* 14:109–116.
43. Harper LR, et al. (2019) Development and application of environmental DNA surveillance for the threatened crucian carp (*Carassius carassius*). *Freshw Biol* 64:93–107.
44. Standley CJ, Dobson AP, Stothard JR (2012) Out of animals and back again: Schistosomiasis as a zoonosis in Africa. *Schistosomiasis*, ed Rokni MB (IntechOpen, Rijeka, Croatia).
45. Sokolow SH, et al. (2018) To reduce the global burden of human schistosomiasis, use “old fashioned” snail control. *Trends Parasitol* 34:23–40.
46. Valentini A, et al. (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol Ecol* 25:929–942.
47. Spens J, et al. (2017) Comparison of capture and storage methods for aqueous microbial eDNA using an optimized extraction protocol: Advantage of enclosed filter. *Methods Ecol Evol* 8:635–645.
48. Sigsgaard EE, Carl H, Møller PR, Thomsen PF (2015) Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. *Biol Conserv* 183:46–52.
49. Jerde CL, Mahon AR, Chadderton WL, Lodge DM (2011) “Sight-unseen” detection of rare aquatic species using environmental DNA. *Conserv Lett* 4:150–157.
50. Shogren AJ, et al. (2017) Controls on eDNA movement in streams: Transport, retention, and resuspension. *Sci Rep* 7:5065.
51. Buxton AS, Groombridge JJ, Zakaria NB, Griffiths RA (2017) Seasonal variation in environmental DNA in relation to population size and environmental factors. *Sci Rep* 7:46294.
52. Goldberg CS, Strickler KM, Fremier AK (2018) Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs. *Sci Total Environ* 633:695–703.
53. Smart AS, et al. (2016) Assessing the cost-efficiency of environmental DNA sampling. *Methods Ecol Evol* 7:1291–1298.
54. Diakité NR, et al. (2017) Dynamics of freshwater snails and *Schistosoma* infection prevalence in schoolchildren during the construction and operation of a multipurpose dam in central Côte d’Ivoire. *Infect Dis Poverty* 6:93.
55. Kazibwe F, et al. (2010) Transmission studies of intestinal schistosomiasis in Lake Albert, Uganda and experimental compatibility of local *Biomphalaria* spp. *Parasitol Int* 59:49–53.
56. Biggs J, et al. (2015) Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biol Conserv* 183:19–28.
57. World Health Organization (2017) *Field Use of Molluscicides in Schistosomiasis Control Programmes: An Operational Manual for Programme Managers* (World Health Organization, Geneva).
58. de Laval F, Savini H, Bianche-Valero E, Simon F (2014) Human schistosomiasis: An emerging threat for Europe. *Lancet* 384:1094–1095.
59. Boissier J, et al. (2015) Schistosomiasis reaches Europe. *Lancet Infect Dis* 15:757–758.
60. Stensgaard A-S, Vounatsou P, Sengupta ME, Utzinger J (2019) Schistosomes, snails and climate change: Current trends and future expectations. *Acta Trop* 190:257–268.
61. Stensgaard A-S, et al. (2013) Large-scale determinants of intestinal schistosomiasis and intermediate host snail distribution across Africa: Does climate matter? *Acta Trop* 128:378–390.
62. Kariuki HC, et al. (2004) Distribution patterns and cercarial shedding of *Bulinus natus* and other snails in the Msambweni area, Coast Province, Kenya. *Am J Trop Med Hyg* 70:449–456.
63. Kariuki HC, et al. (2013) Long term study on the effect of mollusciciding with niclosamide in stream habitats on the transmission of *Schistosomiasis mansoni* after community-based chemotherapy in Makueni district, Kenya. *Parasit Vectors* 6:107.
64. Danish Bilharziasis Laboratory (1987) *A Guide to African Freshwater Snails, 2. East African Species* (Danish Bilharziasis Laboratory, Charlottenlund, Denmark).
65. Schnell IB, et al. (2012) Screening mammal biodiversity using DNA from leeches. *Curr Biol* 22:R262–R263.
66. Borin S, et al. (2008) DNA is preserved and maintains transforming potential after contact with brines of the deep anoxic hypersaline lakes of the Eastern Mediterranean Sea. *Saline Syst* 4:10.
67. Eichmiller JJ, Best SE, Sorensen PW (2016) Effects of temperature and trophic state on degradation of environmental DNA in lake water. *Environ Sci Technol* 50:1859–1867.
68. Strickler KM, Fremier AK, Goldberg CS (2015) Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biol Conserv* 183:85–92.