Methodological assessment for efficient collection of Schistosoma mansoni environmental DNA and improved schistosomiasis surveillance in tropical wetlands

Osawa, Ryosuke ; Jo, Toshiaki S. ; Nakamura, Risa ; Futami, Kyoko ; Itayama, Tomoaki ; Chadeka, Evans Asena ; Ngetich, Benard ; Nagi,…

(Citation)
Acta Tropica,260:107402

(Issue Date)
2024-12

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/0100491617

Highlights

- Rapid surveillance of Schistosoma transmission risk areas is vital.
- Laboratory experiments and field surveys were conducted.
- Targeting >3 μm size fraction was sufficient to capture *S. mansoni* eDNA.
- Sample size and PCR replicates required for its sufficient detection were inferred.
- Performance of eDNA analysis for *S. mansoni* monitoring was fine-tuned.

Title:

Methodological assessment for efficient collection of *Schistosoma mansoni* environmental DNA and improved schistosomiasis surveillance in tropical wetlands

Authors:

Ryosuke Osawa[1*], Toshiaki S Jo[1,2,3*], Risa Nakamura[4,5,6], Kyoko Futami[5,6,7], Tomoaki Itayama[8], Evans Asena Chadeka[4,6,9], Benard Ngetich[9], Sachiyo Nagi[4,10], Mihoko Kikuchi[5,6,11], Sammy M. Njenga[12], Collins Ouma[13], George O. Sonye[14], Shinjiro Hamano[4,5,6,9], and Toshifumi Minamoto[1]

(*R.O. and T.S.J. equally contributed to the work.)

Affiliations:

[1]Graduate School of Human Development and Environment, Kobe University: 3-11, Tsurukabuto, Nada-ku, Kobe, Hyogo, 657-8501, Japan

[2]Research Fellow of Japan Society for the Promotion of Science: 5-3-1 Kojimachi, Chiyoda-ku, Tokyo 102-0083, Japan

[3]Faculty of Advanced Science and Technology, Ryukoku University: 1-5, Yokotani, Oe-cho, Seta, Otsu City, Shiga 520-2194, Japan

[4]Department of Parasitology, Institute of Tropical Medicine (NEKKEN), Nagasaki University: 1-12-4, Sakamoto, Nagasaki, 852-8523, Japan

[5]Program for Nurturing Global Leaders in Tropical and Emerging Communicable Diseases, Graduate School of Biomedical Sciences, Nagasaki University: 1-12-4, Sakamoto, Nagasaki, 852-8523, Japan

[6]The Joint Usage/Research Center on Tropical Disease, Institute of Tropical Medicine (NEKKEN), Nagasaki University: 1-12-4, Sakamoto, Nagasaki, 852-8523, Japan

[7]Department of Vector Ecology and Environment, Institute of Tropical Medicine (NEKKEN), Nagasaki University: 1-12-4, Sakamoto, Nagasaki, 852-8523, Japan

[8]Graduate School of Engineering, Nagasaki University: 1-12-4, Bunkyo-cyo, Nagasaki, 852-8131, Japan

[9]Institute of Tropical Medicine (NUITM), Kenya Medical Research Institute (KEMRI): P O Box 19993-00202, Nairobi, Kenya

[10]Department of Hygiene and Public Health, Tokyo Women's Medical University: 8-1 Kawada-machi, Shinjuku-ku, Tokyo 162-0054, Japan.

[11]Department of Immunogenetics, Institute of Tropical Medicine (NEKKEN), Nagasaki University: 1-12-4, Sakamoto, Nagasaki, 852-8523, Japan

[12]Eastern and Southern Africa Centre of International Parasite Control (ESACIPAC), Kenya Medical Research Institute (KEMRI): P O Box 19993-00202, Nairobi, Kenya

[13]Department of Biomedical Sciences and Technology, School of Public Health and Community Development, Maseno University: Maseno, Kenya

[14]Ability to solve by Knowledge (ASK) Community Based Organization: P.O. Box 30, Mbita, Kenya

Corresponding author:

Toshifumi Minamoto

Graduate School of Human Development and Environment, Kobe University: 3-11, Tsurukabuto, Nada-ku, Kobe City, Hyogo 657-8501, Japan

Tel: +81-78-803-7743

E-mail: minamoto@people.kobe-u.ac.jp

Abstract

Schistosomiasis, caused by trematodes of genus *Schistosoma*, is among the most seriously neglected tropical diseases. Although rapid surveillance of risk areas for *Schistosoma* transmission is vital to control schistosomiasis, the habitat and infection status of this parasite are difficult to assess. Environmental DNA (eDNA) analysis, involving the detection of extra-organismal DNA in water samples, facilitates cost-efficient and sensitive biomonitoring of aquatic environments and is a promising tool to identify *Schistosoma* habitat and infection risk areas. However, in tropical wetlands, highly turbid water causes filter clogging, thereby decreasing the filtration volume and increasing the risk of false negatives. Therefore, in this study, we aimed to conduct laboratory experiments and field surveys in Lake Victoria, Mbita, to determine the appropriate filter pore size for *S. mansoni* eDNA collection in terms of particle size and filtration volume. In the laboratory experiment, aquarium water was sequentially filtered using different pore size filters. Targeting >3 µm size fraction was found to be sufficient to capture *S. mansoni* eDNA particles, regardless of their life cycle stage (egg, miracidia, and cercaria). In the field surveys, GF/D (2.7 µm nominal pore size) filter yielded 2.5-times the filtration volume obtained with a smaller pore size filter and pre-filtration methods under the same time constraints. Moreover, a site-occupancy model was applied to

the field detection results to estimate *S. mansoni* eDNA occurrence and detection probabilities and assess the number of water samples and PCR replicates necessary for efficient eDNA detection. Overall, this study reveals an effective method for *S. mansoni* eDNA detection in turbid water, facilitating the rapid and sensitive monitoring of its distribution and cost-effective identification of schistosomiasis transmission risk areas.

Keywords:

environmental DNA (eDNA); filtration; quantitative real-time PCR (qPCR); *Schistosoma mansoni*; schistosomiasis; site occupancy-detection model (SODM)

1. Introduction

Schistosomiasis is an acute and chronic parasitic disease caused by blood flukes (trematode worms) belonging to genus *Schistosoma*. It has been reported in 78 countries in Africa, Latin America, South America, Middle East, and Southeast Asia, depending on the species (World Health Organization, 2023). At least 251.4 million people required preventive treatment for schistosomiasis in 2021, and deaths and disability-adjusted life years (DALYs) due to schistosomiasis were 10,100 and 1.9 million in 2016, respectively (GBD 2016 Causes of Death Collaborators, 2017; World Health Organization, 2023). Schistosomiasis is among the most serious neglected tropical diseases (NTDs) and has the second-highest socioeconomic impact after malaria among parasitic diseases (Cioli et al., 2014). *Schistosoma* larvae (cercariae) are released from freshwater snails (intermediate hosts), and humans get infected after contact with *Schistosoma*-infested water (Colley et al., 2014). Infected people can further contaminate freshwater sources via feces and urine containing the parasite eggs, which hatch in the water and lead to water-borne infections (Gryseels et al., 2006; World Health Organization, 2023). In the body of definitive hosts, cercariae develop into schistosomulae (immature schistosomes), migrate to the lungs through venous circulation, then to the left heart, and eventually mature into adult schistosomes in the liver, intestine, and urogenital

vessels. Some eggs deposited by adult worms remain in the body tissues and cause immune reactions and progressive damage to organs (Pearce & MacDonald, 2002; McManus et al., 2018).

Schistosomiasis treatment mainly relies on praziquantel, a safe and low-cost anthelmintic agent (Cioli et al., 2014; World Health Organization, 2023). Although praziquantel treatment alleviates the symptoms, patients can get reinfected on repeated contact with contaminated water (Gryseels et al., 2006). Therefore, rapid identification of areas at risk of *Schistosoma* transmission is necessary to develop suitable control strategies for schistosomiasis and protect public health. Distribution of host snails and infection status are routinely used to assess infection risk areas (Gouvras et al., 2017; Kamel et al., 2021), which is essential for the surveillance and control of schistosomiasis. However, this method requires substantial efforts for snail collection and taxonomic expertise for identification. In addition, even in high *Schistosoma* transmission risk areas, infection rate of intermediate hosts is generally low (approximately 1 to 10%) (Wang et al., 2009). Moreover, high fluctuation in water levels during wet seasons and the recent anthropogenic expansion of arable land significantly affect the potential habitats of intermediate host snails and

Schistosoma (Mereta et al., 2019; Oso & Odaibo, 2021), making collection- and observation-based surveys difficult and insufficient for infection control.

Recently, environmental DNA (eDNA) analysis has been widely used to monitor the occurrence and diversity of various macro-organisms, including fish, other vertebrates, and macroinvertebrates, in water samples (Minamoto et al., 2012; Lacoursière-Roussel et al., 2016; Bista et al., 2017; Jo et al., 2020; Sales et al., 2020). The term eDNA refers to all DNA present in the environment, not only the DNA within the living organism but also the DNA released outside the organisms (Pawlowski et al., 2020; Jo et al., 2022). By collecting water samples and analyzing the DNA fragments, eDNA analysis faciltates non-destructive, sensitive, and cost-efficient surveillance of aquatic ecosystems (Darling & Mahon, 2011; Goldberg et al., 2016; Keck et al., 2022). Previous studies have reported the successful detection of *Schistosoma* and other trematode eDNAs from water samples and infected snails as well as their positive correlations with cercariae and infected snail abundances, suggesting eDNA analysis as a promising tool for habitat surveillance and identification of infection risk areas (Hashizume et al., 2017; Sato et al., 2018; Sengupta et al., 2019; Alzaylaee et al., 2020a; 2020b).

Some challenges are associated with the practical application of eDNA analysis for *Schistosoma* surveillance in natural environments. Most studies use a relatively small pore size filter to collect eDNA particles from the water (e.g., 0.22 μm pore size Sterivex-filters [Merck KGaA, Germany] or 0.7 μm nominal pore size glassfiber filters GF/F [GE Healthcare Life Science, UK]) (Sato et al., 2018; Sengupta et al., 2019; Alzaylaee et al., 2020a). However, the optimal filter pore size for collecting *Schistosoma* eDNA remains unknown. Although a smaller pore size filter can capture more eDNA particles, especially in tropical wetlands that is the main habitat of *Schistosoma*, filter clogging due to highly turbid water can decrease the filtration volume and eDNA yield, accumulate PCR inhibitors (e.g., humic, fulvic, and tannic acids), and consequently increase the risk of false-negative detection (Robson et al., 2016; Uchii et al., 2019). Therefore, the state and particle size of *Schistosoma* eDNA should be examined in the water at each life stage to determine suitable filtration strategies for eDNA-based *Schistosoma* surveillance. Most studies on eDNA particle size and optimal filter pore size for eDNA collection have mainly focused on fish species (Turner et al., 2014; Minamoto et al., 2016; Jo et al., 2019; Cooper et al., 2022), with parasites, including *Schistosoma* species, receiving much less attention.

In this study, we conducted laboratory experiments to determine the appropriate filter pore size for *Schistosoma* eDNA collection. We prepared experimental tanks containing *S. mansoni* eggs and miracidia (the life stage after hatching and before the infection of intermediate host snails). We also prepared intermediate host snails (*Biomphalaria glabrata*) infected with *S. mansoni* that released *S. mansoni* cercaria from their bodies. Rearing water samples from the experimental aquaria were sequentially filtered using filters of different pore sizes, and the target eDNA yields were compared with the size fractions for each treatment. Additionally, time-series changes in *S. mansoni* eDNA in intermediate host snails after infection were compared among different size fractions. Based on the results, we conducted field surveys in Lake Victoria, Kenya, to compare the detectability of *S. mansoni* eDNA and filtration volume among different filtration methods and survey the potential distribution of *S. mansoni*. The eDNA field detection results were further analyzed using a multi-scale site occupancy-detection model (SODM), which can estimate target eDNA occurrence (site- and sample-level) and detection (PCR-level) probabilities in relation to various environmental factors (see below). Overall, this study provides a new method for *Schistosoma* eDNA detection in turbid water of typical tropical wetlands.

2. Materials and methods

2.1. Ethics statement

This study was reviewed and approved by the Maseno University Scientific and Ethics Review Committee, Kenya (proposal reference number: MSU/DRPC/MUERC/00056/14) and Ethical Review Board of the Institute of Tropical Medicine, Nagasaki University, Japan (proposal reference number: 10121666).

2.2. Laboratory experiments

The tank experiment was conducted at the Institute of Tropical Medicine of Nagasaki University (Nagasaki Prefecture, Japan) in 2018 and 2019. Seven types of experimental tanks (four replicates per treatment type) were prepared as follows: (i) tanks containing 12,000 eggs of *S. mansoni* (NIH-sm-PR-2-line; egg tank), (ii) tanks containing 12,000 individuals of *S. mansoni* miracidia (miracidia tank), (iii to vi) tanks containing 20 *B. glabrata* snails (Newton's NIH PuertoRican/Brazilian M-line) at 2-, 3-, 4-, and 6-week post-infection (wpi) to five *S. mansoni* miracidia per snail (2-, 3-, 4-, and 6-wpi tanks), and (vii) tanks containing 20 uninfected snails (uninfected tank). We also prepared a tank filled with only 1 L of tap water as a blank tank for each treatment (seven blanks in total). The egg tanks were filled with

1 L of phosphate-buffered saline and shaded in a styrofoam box to prevent the eggs from hatching. To prepare the miracidia tanks, 12,000 eggs were added to tanks filled with 1 L of aged tap water and exposed to light to induce hatching (i.e., *S. mansoni* miracidia and their egg fragments in the miracidia tanks at the time of water sampling). The 2-, 3-, 4-, and 6-wpi and uninfected tanks were filled with 1 L of aged tap water. The room temperature was maintained at 24.2 to 24.9 °C using an air conditioner during the experiments.

The day after tank preparation, water samples were collected from all experimental tanks. After mixing, 250 mL of rearing water was collected using a 50-mL pipette (500 mL water was collected from the uninfected and corresponding blank tanks). Each water sample was placed in a 1-L plastic bottle, and 1 mL of 10% benzalkonium chloride (BAC) was added to suppress eDNA degradation (Jo et al., 2021). The collected water samples were sequentially filtered using a series of 47-mm-diameter polycarbonate membrane filters (10, 3, 0.8, and 0.4 μm pore sizes; MILLIPORE, USA) following Jo et al. (2019). Sequential filtration was performed using a combination of magnetic filter funnels (500 mL capacity; Pall Corporation, USA), plastic holders (ADVANTEC, Japan), nipple joints (ADVANTEC), and hoses (TOYOX, Japan), which divided the eDNA particles into four size fractions (>10, 3–10, 0.8–3, and 0.4–0.8 μm). Throughout the experiment, disposable gloves were worn

when collecting water samples, and the equipment used for water collection and filtration (plastic bottles, filter funnels, plastic holders, nipple joints, hoses, and tweezers) was bleached in 0.1% sodium hypochlorite solution for at least 5 min after every use, followed by rinsing with tap and distilled water (Jo et al., 2019). All filter samples were covered with commercial aluminum foil and stored at –20 °C until DNA extraction.

2.3. Field surveys in Lake Victoria

Field surveys were conducted in Lake Victoria, Mbita (Homa Bay County, Kenya), which has a high incidence of schistosomiasis (Morgan et al., 2001; Nagi et al., 2014; Chadeka et al., 2019), from September 7 to 15, 2018 (Figure 1) during the dry season. Four sites were selected for water sampling: GA1, RB3, GA3, and RA4 (Table S1). At each site, water samples were collected from swamps containing *S. mansoni* (vegetation area; triplicates at GA1 and RB3 and duplicates at GA3 and RA4) and open water areas used by the local people as living water sources (open water area; one replicate at all sites). Surface water (5 L per area) was collected gently to avoid making the water turbid using a 5-L PET bottle, and 5 mL of 10% BAC was added to the water sample to suppress eDNA degradation during transportation to the laboratory at NUITM-KEMRI research station (Kenya Medical Research

Institute in the Nagasaki University Institute of Tropical Medicine) in Mbita. Environmental parameters (water temperature [°C], pH, electrical conductivity [EC; µS/cm], turbidity [mg/L], and dissolved oxygen [DO; mg/L]) were simultaneously measured at each site using a portable multi-parameter water quality meter WQC-24 (DKK-TOA Co., Japan).

The collected water samples were split into five 1-L subsamples and filtered using a hand pump (Hashizume et al., 2017) using the following five methods: (a) filtration with a 47-mm-diameter glassfiber filter with 2.7 µm nominal pore size (GF/D; GE Healthcare Life Science), (b) filtration with a 47-mm-diameter glassfiber filter with 0.7 µm nominal pore size (GF/F), and (c–e) pre-filtration with a 47-mm-diameter nylon net filter with 100, 60, and 20 µm mesh sizes (MILLIPORE), followed by filtration with the GF/F filter. Each water subsample was filtered twice using each method, and each of the two filter samples derived from each subsample was treated individually. The filtration volume was recorded 10 min after the start of filtration due to filter clogging. As a negative control (NC), 500 mL of store-bought bottled water was filtered through a GF/F filter after filtering the field water samples.

Owing to poor detectability in the previous field surveys (see Results), we re-conducted field surveys in Lake Victoria the following year (November 28 to December 3, 2019) during the wet season. In addition to the four sites selected in 2018 (GA1, RB3, GA3,

and RA4), seven new sites were selected for water sampling (GA4, RA6, RA-public, Wath Lwanda Rombo B [site 1], Kaswanga BMU [site 2], Wath Kakimko [site 3], and Wadh Mon Sienga [Site 4], resulting in a total of 11 sites (Figure 1). During the survey period, water sampling was repeated three times at GA1, GA4, RA4, and RA6 and once at the other sites (Table S1). Eight replicates of 1 L water samples were collected using a 1-L PET bottle at each site and sampling time, and 1 mL of 10% BAC was added to the water samples, as described above. Environmental parameters were measured as described above (Table S1). In the laboratory, each water sample was filtered using the GF/D filter, and 500 mL of distilled water was filtered at each site as filtration NC. Final filtration volume was recorded 5 min after the start of filtration due to filter clogging (Table S1).

2.4. Molecular analyses

Total DNA on the polycarbonate membrane and glass fiber (GF/F and GF/D) filters was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Germany; Appendix S1). *S. mansoni* eDNA concentrations in the filtered samples were estimated by quantifying the copy number of mitochondrial cytochrome oxidase I (COI) genes from the target species using the StepOnePlus Real-Time PCR system (Thermo Fisher Scientific, USA). All primers and

TaqMan probes were obtained from Sato et al. (2018). This assay produced 162 bp amplicons derived from mitochondrial COI genes specific to the target species (Sato et al., 2018). The sensitivity of the assay was newly assessed in this study and the limits of detection and quantification (LOD and LOQ, respectively) were estimated as 0.3 and 3 copies, respectively (Appendix S2). Then, 20 µL of TaqMan reaction contained 2 µL of template DNA or pure water (PCR negative control), final 900 nM each of forward and reverse primers, 125 nM of TaqMan probe, and 0.1 µL of Amp Erase Uracil N-Glycosylase (Thermo Fisher Scientific) in 1 × TaqMan Environmental Master Mix 2.0 (Thermo Fisher Scientific). The thermal conditions for quantitative real-time-PCR (qPCR) were as follows: 2 min at 50 °C, 10 min at 95 °C, and 55 cycles of 15 s at 95 °C and 1 min at 60 °C. A dilution series of *S. mansoni* COI amplicons was used as a quantification standard and run simultaneously to estimate the copy number of the target eDNA in the sample (Appendix S2). All qPCRs were performed in triplicates and included eDNA samples, dilution series, blank samples, and negative controls. The eDNA concentration in each sample was calculated as the mean of triplicate measurements, and each PCR-negative replicate (indicating non-amplification) was considered to contain 0 copies (Jo et al., 2021). However, all samples in the field surveys in

both years exhibited extremely low eDNA concentrations (< 0.1 copies per template DNA); therefore, only detection/non-detection per PCR replicate was considered.

We also checked PCR inhibition in samples collected from Lake Victoria in 2018 using an internal positive control. Lambda phage DNA and real-time PCR assay were used (Minamoto et al., 2009; Honjo et al., 2010). Briefly, 20 µL of TaqMan reaction contained 2 µL of eDNA extract, final 900 nM each of forward and reverse primers, 125 nM of TaqMan probe, 0.1 µL of Amp Erase Uracil N-Glycosylase, and Lambda phage DNA (3,000 copies per PCR reaction) or pure water (standard) in 1 × TaqMan Environmental Master Mix 2.0. Thermal conditions for qPCR were previously described by Honjo et al. (2010). All qPCRs, including spike, standard samples, and PCR negative controls (similar to the procedure described above), were performed in duplicate. The eDNA sample was considered to be PCR-inhibited if ΔCt (difference in Ct values of each spiking sample from that of the standard sample) > 2, as previously reported (Jo et al., 2020). All GA1 samples and approximately half of the samples collected from the other three sites (92 samples in total) were tested for PCR inhibition (Table S2).

2.5. Statistical analyses

All statistical analyses were performed using R version 4.2.2 (R Core Team, 2023). In the laboratory experiments, all eDNA concentrations per PCR reaction (copies/2 µL template DNA) were converted into those per water sample (copies/mL water). First, to compare the target eDNA concentrations at different size fractions (>10, 3–10, 0.8–3, and 0.4–0.8 µm; as a categorical variable), linear mixed models (LMMs) and post-hoc multiple comparison tests were performed for each treatment type using the packages, *lmerTest* (Kuznetsova et al., 2017) and *multcomp* (Hothorn et al., 2008). The eDNA concentrations (x) in all samples were subjected to $\log10(x + 0.1)$ transformation to meet normality, as the target eDNA was not detected in some samples in the 2-wpi tanks. Different tank IDs were considered random effects. Second, target eDNA concentrations in each size fraction (>10, 3–10, 0.8–3, and 0.4–0.8 µm) were converted to a proportion of total sequential filtration for each experimental tank. Third, generalized linear models (GLMs) with Gaussian distribution were used to assess the changes in the target eDNA concentrations (log10-transformed) and proportions for each size fraction after infection with intermediate snails (*B. glabrata*; 2, 3, 4, and 6-wpi; as a categorical variable).

In the 2018 field survey, a generalized linear mixed model (GLMM) with a gamma distribution, followed by a post-hoc multiple comparison test, was used to compare the

filtration volume recorded 10 min after the start of filtration in the five filtration methods using the packages, *lmerTest* and *multcomp*. Survey sites (GA1, RB3, GA3, and RA4), areas (vegetation and open water; nested within survey sites), and filtration replicates (nested within areas) were hierarchically considered as random effects.

In the 2019 field survey, SODM with the Bayesian Monte Carlo Markov Chain (MCMC) algorithm was performed using the package, *eDNAoccupancy* (Dorazio & Erickson, 2018). Based on the eDNA detection result, the following probabilities were estimated for each survey site: Ψ (site-level; probability of target eDNA occurrence at a given sampling site), θ (water sample-level; conditional probability of target eDNA occurrence in each sample at a given site when the target eDNA is present at that site), and ρ (PCR replicate-level; conditional probability of the target eDNA detection in each PCR replicate of a given eDNA sample when the target eDNA is present in the sample) (Dorazio & Erickson, 2018; Sengupta et al. 2019; Jo et al., 2020). According to the default settings, 10,000 iterations of the MCMC algorithm were run per model, and the burn-in was set at 1,000 to discard the initial transient region of the chain to obtain precise parameter estimates.

Prior to SODM, mean values of water temperature, pH, EC, turbidity, DO, and infection rate of humans with *S. mansoni* (Appendix S3) at each survey site were calculated to

construct a Pearson's correlation matrix with Bonferroni's correction (significance level: $\alpha = 0.05/{}_7C_2 \fallingdotseq 0.0024$), and high levels of correlations were detected between pH and DO ($r = 0.974$; $P < 0.001$), pH and temperature ($r = 0.825$; $P = 0.0018$), DO and temperature ($r = 0.803$; $P = 0.0029$), and turbidity and filtration volume ($r = –0.812$; $P = 0.0024$). Therefore, these variables were omitted, and only EC, filtration volume, and infection rate were considered as factors affecting each probability in the SODM. Infection rate was included in Ψ assuming that more *S. mansoni* eDNA may be present at the site with a high infection rate. Filtration volume was included in θ assuming that low filtration volume decreases the capture efficiency of the target eDNA particles. EC was included in ρ assuming that high EC indicates more amount of PCR inhibitory substances, such as humic and tannic acids, at the site (Uchii et al., 2019).

Based on each of the probabilities in SODM, the cumulative availability probability θ^* in relation to the number of water samples n (when the target eDNA was present at the site) and cumulative detection probability ρ^* in relation to the number of PCR replicates m (when the target eDNA was present in the sample) were estimated using the following equations (Sengupta et al., 2019; Jo et al., 2020):

$$\theta^* = 1 - (1 - \bar{\theta})^n$$

$$\rho^* = 1 - (1 - \bar{\rho})^m$$

where $\bar{\theta}$ is the mean value of the sample-level occurrence probability at all survey sites, and $\bar{\rho}$ is the mean value of the PCR replicate-level detection probability at all survey sites.

3. Results

3.1. Laboratory experiments

In real-time PCR, R^2 value and PCR efficiency were 0.993 to 1.000 and 86.0 % to 89.8%, respectively. Although some blank samples showed PCR amplification, their concentrations ranged from 0.1 to 1.4 copies in the water and were much lower than those in the corresponding tank samples (Table S2). In addition, one sample in the uninfected tank also showed PCR amplification (approximately 0.1 copies per mL water; Table S2) possibly due to inter-sample contamination. None of the PCR negative controls was amplified throughout the experiment.

LMMs and post-hoc comparison tests showed that *S. mansoni* eDNA concentrations were significantly higher at >10 μm size fraction and significantly lower at 0.4–0.8 μm size fraction than at the other size fractions for all treatments ($P < 0.01$), except for the 2-wpi tanks (Table S3; Figure 2). Approximately 98.3 and 99.6% of *S. mansoni* eDNA were detected

from >10 µm size fractions in the egg and miracidia tanks, respectively (Table 1). Moreover, most of the target eDNA was detected from >10 µm size fractions in the 3-, 4-, and 6-wpi tanks (73.8, 67.2, and 90.4% on average, respectively). In contrast, in the 2-wpi tanks, the target eDNA concentrations were significantly lower than those in the other treatments, and the differences in eDNA concentrations among different size fractions were unclear (Table 1; Figure 2). These findings were also supported by the GLMs, which showed that the target eDNA concentrations were significantly lower in the 2-wpi tanks than in the 3-, 4-, and 6-wpi tanks for all size fractions ($P < 0.001$; Figure 3). In contrast, post-infection, the proportion of *S. mansoni* eDNA derived from intermediate snails significantly increased in the >10 µm size fraction (GLM; $P < 0.05$) but decreased in the smaller size fractions over time (Figure 3; Table S3).

3.2. Field surveys in Lake Victoria

In the 2018 field survey, *S. mansoni* eDNA was only detected in five subsamples at GA1, but not at the other sites (Table S2). In the inhibition test, ΔCt values ranged from –0.3 to 0.3 and PCR inhibition effect was negligible (Table S2). Water temperature, pH, EC, turbidity, and DO were 25.8 ± 1.1 °C, 7.2 ± 0.7, 252.1 ± 145.1 µS/cm, 84.8 ± 60.8 mg/L, and 10.9 ± 21.3

mg/L (mean ± standard deviation [SD]), respectively. In addition, GLMM showed that the water filtration volume was significantly higher in GF/D filtration than in the other filtration methods ($P < 0.001$; Table S4; Figure S1).

In the 2019 field survey, we only used the GF/D filter for water filtration based on the above results, including the laboratory experiment results. *S. mansoni* eDNA was detected in 16 samples collected from GA1 (eight on Nov 28, four on Nov 30, and four on Dec 3) and eight samples collected from GA4 (all on Dec 3), but not at the other sites (Table S2). In SODM, Ψ was high at sites with high infection rates, θ increased with increasing filtration volume, and ρ was lower at sites with high EC, although uncertainties were high for Ψ and θ (i.e., 95% confidence intervals [CIs] of the covariates encompassed zero; Table 2; Figure 4). The median estimates of Ψ, θ, and ρ at each survey site were 0.025–0.384 (0.212 on average), 0.391–0.926 (0.788 on average), and 0.610–0.978 (0.915 on average), respectively. As long as the target eDNA was present at a specific site or in a specific sample, θ^* and ρ^* were estimated to exceed 0.95, including their 95% CIs ($\bar{\theta}$: 0.390–0.913; $\bar{\rho}$: 0.784–0.974), when more than seven water samples were collected from the site and more than two PCR replicates were prepared from the collected water samples (Figure S2).

4. Discussion

4.1. Laboratory experiments

We found that a large proportion of *S. mansoni* eDNA derived from its eggs, miracidia, and cercaria generally existed in the >10 µm size fraction. As all eggs, miracidia, and cercaria are tens to hundreds of µm in diameter (Xu & Dresden, 1989; Sengupta et al., 2019), eDNA detected in > 10 µm size fraction may be derived from *S. mansoni* eggs or the individuals themselves, and their extra-organismal DNA (true eDNA) may be detected in the smaller size fractions. In the egg and miracidia tanks, proportions of true eDNA (eDNA detected in <10 µm size fraction) were less than 2%. Notably, egg-derived eDNA (not the egg itself) shows a very low concentration, which may be undetectable in other taxa, such as fish and amphibians (Takeuchi et al., 2019; Takeshita et al., 2020). Here, proportion of true eDNA in the tanks with infected intermediate host snails (i.e., cercaria-derived eDNA) was 10–30% (except for the 2-wpi tanks; discussed below). Therefore, *S. mansoni* eDNA is mainly detected in the whole organisms, eggs, and extra-organismal cercaria DNA. Sengupta et al. (2019) also reported that *S. mansoni* eDNA is detectable from tank water even if whole cercariae are removed from the tank via pre-filtration with a 12-µm mesh size filter, but the results may vary by 2–3 orders of magnitude in the presence and absence of cercariae.

Cercariae are non-feeding stages with a short-lived ecology (from 10 hours to 3 days) (Schreiber et al., 1949). A previous study revealed an approach to collect cercariae using a large pore size filter to detect the organismal DNA (Hung & Remais, 2008); however, this approach increases the likelihood of non-detection due to death or decomposition, leading to an uncertain and biased diagnosis. Additionally, cercaria-based approach requires a relatively large volume of water using a zooplankton net, which is expensive and its reuse requires decontamination between sampling events (Alzaylaee et al., 2020a). On the contrary, extra-organismal DNA (true eDNA) persists in water after decomposition (from days to weeks, depending on the environmental conditions) (Jo & Minamoto, 2021), facilitaing more sensitive and precise surveillance (Hashizume et al., 2017; Sengupta et al., 2019; Kamel et al., 2021). In our experimental tanks with infected intermediate host snails, 83.4–96.4% of the target eDNA on average was detected in the > 3 µm size fraction and 98.7–99.8% of the target eDNA on average was detected in the > 0.8 µm size fraction (except for the 2-wpi tanks). Therefore, targeting eDNA particles in the >3 µm size fraction is a practical approach to capture both *S. mansoni* and its eDNA with reduced filter clogging risk for samples collected from turbid environments.

Compared to the other treatments, *S. mansoni* eDNA concentrations were significantly lower in the 2-wpi tanks in all size fractions. As the experimental tanks had a higher individual density (20 infected snails per L of water) than the natural environments, the cercariae themselves and their extra-organismal DNAs released from intermediate host snails were hardly detected in the early infection stages (approximately two weeks post-infection) in the field. In contrast, the amount of *S. mansoni* eDNA drastically increased in all size fractions over the next week (from 2-wpi to 3-wpi), indicating that it takes three weeks or longer for *S. mansoni* cercariae to mature and escape from host snails. As the infection progressed, the proportion of target eDNA in the >10 μm size fraction (possibly cercariae themselves) gradually increased. Accordingly, the proportion of true *S. mansoni* eDNA detected in the smaller fractions decreased. These results confirm a previous report that *S. mansoni* propagates via asexual reproduction in intermediate host snails (Colley et al., 2014).

4.2. Field surveys in Lake Victoria

The advantage of targeting eDNA particles at >3 μm size fraction was reinforced by the field survey in 2018 in terms of filtration volume. In our laboratory experiments, eDNA detection rate and yield per filtration volume were high for filters with small pore sizes. However, in

field surveys, in addition to the risk of filter clogging, the time required for filtration must be considered for survey accuracy. Although the target eDNA was not detected in most of the water samples, use of the GF/D (2.7 µm nominal pore size) filter yielded approximately 2.5-times the filtration volume obtained with the filtration methods under the same time constraint (10 min per filtration). Pre-filtration methods have been used as alternatives to filters with large pore sizes to avoid filter clogging (Robson et al., 2016; Takasaki et al., 2021). Although no significant difference was observed in the filtration volume with the different pre-filtration methods, the filtration volume with any of tested the pre-filtration methods was less than that obtained with the GF/D filter. In the survey area, most suspended particles causing filter clogging were smaller than 20 µm in diameter (corresponding to the minimum pore size of the pre-filtration filter). These results indicate that a high eDNA detection rate and yield can be achieved using the GF/D filter if *S. mansoni* eDNA is detected in the study area.

Factors related to intermediate host snail density and infectivity were possibly responsible for the severely low detection rate of *S. mansoni* eDNA in the 2018 survey. Snail density and infectivity are affected by various environmental factors, such as pH, conductivity, and human activity (Rowel et al., 2015; Mereta et al., 2019). They also change seasonally and are affected by fluctuations in precipitation and water temperature. For

example, *S. mansoni* infection of the intermediate host snail (*B. glabrata*) is positively correlated with rainfall (Calasans et al., 2018). The period of the 2018 survey corresponded to the dry season, in which snail density is generally high (Odongo-Aginya et al., 2008; Adediran et al., 2014; Andrus et al., 2023); however, the detected infection rate was low. This low detection rate of *S. mansoni* eDNA in the dry season was possibly due to seasonality and relatively low quantity of target eDNA in the samples, but not due to PCR inhibition (see Results).

In the following year, survey was performed during the wet season, resulting in an increase in the number of positive samples in GA1, where the target eDNA was also detected in the previous year. *S. mansoni* eDNA was also detected in GA4, where no samples were collected in the previous year. Although human infection with *S. mansoni* was confirmed at all survey sites. the proportion of positive samples was still very low. This low detection frequency largely corresponds to the results of previous studies targeting trematode eDNA in the field (Hashizume et al., 2017; Sato et al., 2018; Sengupta et al., 2019; Alzaylaee et al., 2020a), implying a high risk of false-negative detection in eDNA-based monitoring of trematode parasites, including *S. mansoni*. Here, SODM offered insight into the results of field surveys. ρ was generally high and negatively correlated with EC. The number of PCR

replicates used in this study (triplicate per sample) was similar to that in most eDNA studies for species-specific detection (Doi et al., 2021) and was sufficient to detect the target eDNA as long as it was present in the sample. Notably, this study used the Environmental Master Mix, a PCR reagent resistant to inhibition (Sengupta et al., 2019; Uchii et al., 2019), which may be adequate for eDNA detection in highly turbid environments, such as tropical wetlands.

θ was not as high as ρ, and the increase in eDNA capturability (i.e., cumulative availability probability) with increasing filtration volume was non-significant. The number of water samples necessary to sufficiently capture the target eDNA in this study (seven per site) was much higher than that used in most eDNA studies (one to three; Takahashi et al., 2023). Consistent with our findings, Sengupta et al. (2019) also used SODM and reported that more than seven 1-L water samples are necessary to achieve >95% detection probability of *S. mansoni* eDNA at the sample level, highlighting the importance of a sufficient number of sample replicates per site for sensitive and reliable schistosomiasis surveillance via eDNA analysis. Ψ was low in all survey sites regardless of the infection rate of humans with *S. mansoni*. Use of water sources contaminated with *S. mansoni* and infected intermediate host snails is the main factor for schistosomiasis transmission in the local population. However,

low Ψ estimates in SODM in this study indicate that, even if schistosomiasis transmission to the local population is evident, *S. mansoni* eDNA exhibits a very low concentration and heterogeneous distribution in the field, possibly yielding false negative results. For example, diffusion and dilution effects of water level fluctuations during the wet season weaken the association between eDNA detection and organism occurrence (Yates et al., 2019; Curtis et al., 2021). Diffusion effect may also yield false positives for eDNA detection in sites without *S. mansoni* (Jo et al., 2020). To compensate for the low site-level occupancy probability of *S. mansoni* eDNA and more accurately identify areas at risk for schistosomiasis transmission, replicated water sampling of multiple sites is crucial. Addressing these challenges is necessary for effective and reliable eDNA-based schistosomiasis surveillance. Nevertheless, conventional xeno-monitoring of trematode worms and intermediate hosts is also reliable detection.

4.3. Conclusions and future perspectives

In summary, this study outlined an effective method for *S. mansoni* eDNA detection in turbid water based on laboratory experiments and field surveys. Our findings suggest that targeting eDNA particles >3 μm size fraction is a practical approach to capture not only *S. mansoni*

individuals but also their extra-organismal DNA with reduced risk of filter clogging in a turbid environment. We also used the above filtration method to survey *S. mansoni* eDNA distribution in tropical wetlands in Lake Victoria and estimate its occurrence and detection probabilities at each hierarchical level based on an SODM. The model estimated the number of water samples necessary to capture *S. mansoni* eDNA and number of PCR replicates necessary for its sufficient detection. Our findings can aid in the rapid and sensitive monitoring of *S. mansoni* and other trematode parasites. Additionally, this study fills the knowledge gap related to filtration performance in water types with different turbidities and outlines a specific method to fine-tune pre-PCR sample processing for eDNA analysis (Kumar et al., 2022; Holmes et al., 2024).

For precise assessment of the schistosomiasis transmission risk areas, distribution of *Schistosoma* parasites and their intermediate/definitive hosts must be simultaneously examined as *Schistosoma* life cycle cannot be completed without the host. Although some freshwater snails, such as *Biomphalaria*, and mammals, including humans, are potential intermediate and definitive hosts of *Schistosoma* (He et al., 2001; Morgan et al., 2001), the types of species infected by these parasites and incorporated into their life cycles are difficult to assess in different environments. Sato et al. (2019) performed riverine eDNA

metabarcoding to detect *Leptospira*, a non-pathogenic microbiome, and vertebrates in northern Okinawa, Japan. They showed high correlations in the eDNA occurrence of *Leptospira* and some bacterial and vertebrate species suspected as potential leptospiral reservoirs. Therefore, the eDNA signal itself cannot directly indicate the infection status of the host; however, eDNA analysis can be used as an alternative approach to determine the candidate intermediate/definitive hosts of trematode parasites, including *Schistosoma*, in natural ecosystems. We believe that further exploration of eDNA applications for schistosomiasis surveillance under various environmental and epidemiological conditions will help to prevent schistosomiasis transmission in tropical areas by providing insights on infectious disease ecology, thereby facilitating the development of suitable medical, epidemiological, and sanitary interventions (Liang et al., 2007; Garchitorena et al., 2017; Bass et al., 2023).

Acknowledgements

We would like to thank the ASK Community-Based Organization staff in Mbita, Kenya, for assisting with water collection and water quality measurement, providing experimental space, arranging transportation, and negotiating with the local community. We would also like to thank the NUITM-KEMRI project in Nairobi, Kenya, for providing research permission and making travel arrangements, including accommodation and transportation, and Megumi Hamasaki (Nagasaki University) for maintaining the infected snails used in the laboratory

experiment. This study was supported by JSPS-KAKENHI (grant numbers JP17H01684 and JP21H04852), Grant-in-Aid for JSPS Research Fellows (JP18J20979, JP22J00439, and JP22KJ3043), and International Collaborative Research Program: Science and Technology Research Partnership for Sustainable Development (SATREPS) by JICA and AMED (JP23jm0110027). The manuscript was proofread using Grammarly (https://app.grammarly.com) and Editage (editage.com).

Declaration of competing interest

There is no conflict of interest to declare.

Data accessibility

The raw data of real-time PCR experiments are provided in the Supplemental Information.

Supplemental information

Appendix S1. Detailed information on DNA extraction methods from filter samples.

Appendix S2. Detailed information on preparing a quantification standard for *S. mansoni* eDNA and estimating the LOD/LOQ of the eDNA assay.

Appendix S3. Detailed information on the estimation of human *S. mansoni* infection rate.

Table S1. Detailed information on the field survey in 2019.

Table S2. Raw real-time PCR data in this study.

Table S3. Summary of the LMMs and GLMs for the laboratory experiment.

Table S4. Summary of the GLMM for the field survey in 2018.

Figure S1. Comparison of filtration volume between the five filtration methods in the field survey in 2018.

Figure S2. Cumulative availability probability (θ^*) detection probability (ρ^*) of *S. mansoni* eDNA based on the SODM.

References

Adediran, O. A. (2014). Trematode Infections in Freshwater Snails and Rainfall Patterns in Ibadan North Local Government Area, Oyo State, Nigeria. Journal of Science Research, 13(1), 6-6.

Alzaylaee, H., Collins, R. A., Rinaldi, G., Shechonge, A., Ngatunga, B., Morgan, E. R., Genner, M. J. (2020a) *Schistosoma* species detection by environmental DNA assays in African freshwaters. PLOS Neglected Tropical Diseases, 14(3), e0008129.

Alzaylaee, H., Collins, R. A., Shechonge, A., Ngatunga, B. P., Morgan, E. R., & Genner, M. J. (2020b). Environmental DNA-based xenomonitoring for determining *Schistosoma* presence in tropical freshwaters. Parasites & Vectors, 13(1), 1-11.

Andrus, P. S., Stothard, J. R., & Wade, C. M. (2023). Seasonal patterns of *Schistosoma mansoni* infection within *Biomphalaria* snails at the Ugandan shorelines of Lake Albert and Lake Victoria. PLoS Neglected Tropical Diseases, 17(8), e0011506.

Bass, D., Christison, K. W., Stentiford, G. D., Cook, L. S., & Hartikainen, H. (2023). Environmental DNA/RNA for pathogen and parasite detection, surveillance, and ecology. Trends in Parasitology, 39(4), 285-304.

Bista, I., Carvalho, G. R., Walsh, K., Seymour, M., Hajibabaei, M., Lallias, D., ... & Creer, S. (2017). Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. Nature Communications, 8, 14087.

Calasans, T. A. S., Souza, G. T. R., Melo, C. M., Madi, R. R., & Jeraldo, V. D. L. S. (2018). Socioenvironmental factors associated with *Schistosoma mansoni* infection and intermediate hosts in an urban area of northeastern Brazil. PLoS ONE, 13(5), e0195519.

Chadeka, E. A., Nagi, S., Cheruiyot, N. B., Bahati, F., Sunahara, T., Njenga, S. M., & Hamano, S. (2019). A high-intensity cluster of *Schistosoma mansoni* infection around Mbita causeway, western Kenya: a confirmatory cross-sectional survey. Tropical medicine and health, 47, 26.

Cioli, D., Pica-Mattoccia, L., Basso, A., & Guidi, A. (2014). Schistosomiasis control: praziquantel forever? Molecular and Biochemical Parasitology, 195(1), 23-29.

Colley, D. G., Bustinduy, A. L., Secor, W. E., & King, C. H. (2014). Human schistosomiasis. The Lancet, 383 (9936), 2253-2264.

Cooper, M. K., Villacorta-Rath, C., Burrows, D., Jerry, D. R., Carr, L., Barnett, A., ... & Simpfendorfer, C. A. (2022). Practical eDNA sampling methods inferred from particle size distribution and comparison of capture techniques for a Critically Endangered elasmobranch. Environmental DNA, 4(5), 1011-1023.

Curtis, A. N., Tiemann, J. S., Douglass, S. A., Davis, M. A., & Larson, E. R. (2021). High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. Diversity and Distributions, 27(10), 1918-1931.

Darling, J. A., & Mahon, A. R. (2011). From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments. Environmental Research, 111 (7), 978-988.

Dorazio, R. M., & Erickson, R. A. (2018). EDNAOCCUPANCY: An r package for multiscale occupancy modelling of environmental DNA data. Molecular Ecology Resources, 18(2), 368-380.

Garchitorena, A., Sokolow, S. H., Roche, B., Ngonghala, C. N., Jocque, M., Lund, A., ... & De Leo, G. A. (2017). Disease ecology, health and the environment: a framework to account for ecological and socio-economic drivers in the control of neglected tropical diseases. Philosophical Transactions of the Royal Society B: Biological Sciences, 372(1722), 20160128.

GBD 2016 Causes of Death Collaborators. (2017). Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet, 390 (10100), 1151-1210.

Goldberg, C. S., Turner, C. R., Deiner, K., Klymus, K. E., Thomsen, P. F., Murphy, M. A., ... & Taberlet, P. (2016). Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods in Ecology and Evolution, 7(11), 1299-1307.

Gouvras, A. N., Allan, F., Kinung'hi, S., Rabone, M., Emery, A., Angelo, T., ... & Rollinson, D. (2017). Longitudinal survey on the distribution of *Biomphalaria sudanica* and *B. choanomophala* in Mwanza region, on the shores of Lake Victoria, Tanzania: implications for schistosomiasis transmission and control. Parasites & Vectors, 10, 1-14.

Gryseels, B., Polman, K., Clerinx, J., & Kestens, L. (2006). Human schistosomiasis. The Lancet, 368(9541), 1106-1118.

Hashizume, H., Sato, M., Sato, M. O., Ikeda, S., Yoonuan, T., Sanguankiat, S., ... & Minamoto, T. (2017). Application of environmental DNA analysis for the detection of *Opisthorchis viverrini* DNA in water samples. Acta Tropica, 169, 1-7.

He, Y. X., Salafsky, B., & Ramaswamy, K. (2001). Host–parasite relationships of *Schistosoma japonicum* in mammalian hosts. Trends in parasitology, 17(7), 320-324.

Holmes, A. E., Baerwald, M. R., Rodzen, J., Schreier, B. M., Mahardja, B., & Finger, A. J. (2024). Evaluating environmental DNA detection of a rare fish in turbid water using field and experimental approaches. PeerJ, 11, e16453.

Honjo, M. N., Minamoto, T., Matsui, K., Uchii, K., Yamanaka, H., Suzuki, A. A., ... & Kawabata, Z. (2010). Quantification of cyprinid herpesvirus 3 in environmental water by using an external standard virus. Applied and Environmental Microbiology, 76(1), 161-168.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. Biometrical Journal: Journal of Mathematical Methods in Biosciences, 50(3), 346-363.

Hung, Y. W., & Remais, J. (2008). Quantitative detection of *Schistosoma japonicum* cercariae in water by real-time PCR. PLoS Neglected Tropical Diseases, 2(11), e337.

Jo, T., Arimoto, M., Murakami, H., Masuda, R., & Minamoto, T. (2019). Particle size distribution of environmental DNA from the nuclei of marine fish. Environmental Science & Technology, 53(16), 9947-9956.

Jo, T., & Minamoto, T. (2021). Complex interactions between environmental DNA (eDNA) state and water chemistries on eDNA persistence suggested by meta-analyses. Molecular Ecology Resources, 21(5), 1490-1503.

Jo, T., Sakata, M.K., Murakami, H., Masuda, R., & Minamoto, T. (2021). Universal performance of benzalkonium chloride for the preservation of environmental DNA in seawater samples. Limnology and Oceanography: Methods, 19(11), 758–768.

Jo, T., Takao, K., & Minamoto, T. (2022). Linking the state of environmental DNA to its application for biomonitoring and stock assessment: Targeting mitochondrial/nuclear genes, and different DNA fragment lengths and particle sizes. Environmental DNA, 4(2), 271-283.

Jo, T., Tomita, S., Kohmatsu, Y., Osathanunkul, M., Ushimaru, A., & Minamoto, T. (2020). Seasonal monitoring of Hida salamander *Hynobius kimurae* using environmental DNA with a genus-specific primer set. Endangered Species Research, 43, 341-352.

Kamel, B., Laidemitt, M. R., Lu, L., Babbitt, C., Weinbaum, O. L., Mkoji, G. M., & Loker, E. S. (2021). Detecting and identifying *Schistosoma* infections in snails and aquatic habitats: A systematic review. PLoS Neglected Tropical Diseases, 15(3), e0009175.

Keck, F., Blackman, R. C., Bossart, R., Brantschen, J., Couton, M., Hürlemann, S., ... & Altermatt, F. (2022). Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. Molecular Ecology, 31(6), 1820-1835.

Kumar, G., Farrell, E., Reaume, A. M., Eble, J. A., & Gaither, M. R. (2022). One size does not fit all: Tuning eDNA protocols for high-and low-turbidity water sampling. Environmental DNA, 4(1), 167-180.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. (2017). *lmerTest* package: tests in linear mixed effects models. Journal of Statistical Software, 82, 1-26.

Lacoursière-Roussel, A., Côté, G., Leclerc, V., & Bernatchez, L. (2016). Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. Journal of Applied Ecology, 53(4), 1148-1157.

Liang, S., Seto, E. Y., Remais, J. V., Zhong, B., Yang, C., Hubbard, A., ... & Spear, R. C. (2007). Environmental effects on parasitic disease transmission exemplified by schistosomiasis in western China. Proceedings of the National Academy of Sciences, 104(17), 7110-7115.

McManus, D. P., Dunne, D. W., Sacko, M., Utzinger, J., Vennervald, B. J., & Zhou, X. (2018). Schistosomiasis. Nature Reviews Disease Primers, 4, 13.

Mereta, S. T., Bedewi, J., Yewhalaw, D., Mandefro, B., Abdie, Y., Tegegne, D., ... & Kloos, H. (2019). Environmental determinants of distribution of freshwater snails and trematode infection in the Omo Gibe River Basin, southwest Ethiopia. Infectious Diseases of Poverty, 8, 93.

Minamoto, T., Honjo, M. N., Uchii, K., Yamanaka, H., Suzuki, A. A., Kohmatsu, Y., ... & Kawabata, Z. (2009). Detection of cyprinid herpesvirus 3 DNA in river water during and after an outbreak. Veterinary Microbiology, 135(3-4), 261-266.

Minamoto, T., Naka, T., Moji, K., & Maruyama, A. (2016). Techniques for the practical collection of environmental DNA: filter selection, preservation, and extraction. Limnology, 17, 23-32.

Minamoto, T., Yamanaka, H., Takahara, T., Honjo, M. N., & Kawabata, Z. (2012). Surveillance of fish species composition using environmental DNA. Limnology, 13, 193-197.

Morgan, J. A. T., Dejong, R. J., Snyder, S. D., Mkoji, G. M., & Loker, E. S. (2001). *Schistosoma mansoni* and *Biomphalaria*: past history and future trends. Parasitology, 123(7), 211-228.

Nagi, S., Chadeka, E. A., Sunahara, T., Mutungi, F., Justin, Y. K. D., Kaneko, S., ... & Hamano, S. (2014). Risk factors and spatial distribution of *Schistosoma mansoni* infection among primary school children in Mbita District, Western Kenya. PLoS Neglected Tropical Diseases, 8(7), e2991.

Odongo-Aginya, E. I., Kironde, F. K., Kabatereine, N. B., Kategere, P., & Kazibwe, F. (2008). Effect Of Seasonal Rainfall And Other Environmental Changes, On Snail Density And Infection Rates With *Schistosoma mansoni* Fifteen Years After The Last Snails' Study In Kigungu, Entebbe, Uganda. East African Medical Journal, 85(11), 556-563.

Oso, O. G., & Odaibo, A. B. (2021). Land use/land cover change, physico-chemical parameters and freshwater snails in Yewa North, Southwestern Nigeria. PLoS ONE, 16(2), e0246566.

Pawlowski, J., Apothéloz-Perret-Gentil, L., & Altermatt, F. (2020). Environmental DNA: What's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. Molecular Ecology, 29(22), 4258-4264.

Pearce, E. J., & MacDonald, A. S. (2002). The immunobiology of schistosomiasis. Nature Reviews Immunology, 2(7), 499.

R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Robson, H. L., Noble, T. H., Saunders, R. J., Robson, S. K., Burrows, D. W., & Jerry, D. R. (2016). Fine-tuning for the tropics: application of eDNA technology for invasive fish detection in tropical freshwater ecosystems. Molecular Ecology Resources, 16(4), 922-932.

Rowel, C., Fred, B., Betson, M., Sousa-Figueiredo, J. C., Kabatereine, N. B., & Stothard, J. R. (2015). Environmental epidemiology of intestinal schistosomiasis in Uganda: population dynamics of Biomphalaria (Gastropoda: Planorbidae) in Lake Albert and Lake Victoria with observations on natural infections with digenetic trematodes. BioMed Research International, 2015(1), 717261.

Sales, N. G., McKenzie, M. B., Drake, J., Harper, L. R., Browett, S. S., Coscia, I., ... & McDevitt, A. D. (2020). Fishing for mammals: Landscape-level monitoring of terrestrial and semi-aquatic communities using eDNA from riverine systems. Journal of Applied Ecology, 57(4), 707-716.

Sato, Y., Mizuyama, M., Sato, M., Minamoto, T., Kimura, R., & Toma, C. (2019). Environmental DNA metabarcoding to detect pathogenic *Leptospira* and associated organisms in leptospirosis-endemic areas of Japan. Scientific Reports, 9, 6575.

Sato, M. O., Rafalimanantsoa, A., Ramarokoto, C., Rahetilahy, A. M., Ravoniarimbinina, P., Kawai, S., Minamoto T., Sato, M., Kirinoki, M., Rasolofo, V., De Calan, M., & Chigusa, Y., (2018). Usefulness of environmental DNA for detecting *Schistosoma mansoni* occurrence sites in Madagascar. International Journal of Infectious Diseases, 76, 130-136.

Schreiber, F. G., & Schubert, M. (1949). Experimental infection of the snail *Australorbis glabratus* with the trematode *Schistosoma mansoni* and the production of cercariae. The Journal of Parasitology, 35(1), 91-100.

Sengupta, M. E., Hellström, M., Kariuki, H. C., Olsen, A., Thomsen, P. F., Mejer, H., ... & Vennervald, B. J. (2019). Environmental DNA for improved detection and environmental surveillance of schistosomiasis. Proceedings of the National Academy of Sciences, 116(18), 8931-8940.

Takahashi, M., Saccò, M., Kestel, J. H., Nester, G., Campbell, M. A., Van Der Heyde, M., ... & Allentoft, M. E. (2023). Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution. Science of the Total Environment, 873, 162322.

Takasaki, K., Aihara, H., Imanaka, T., Matsudaira, T., Tsukahara, K., Usui, A., ... & Doi, H. (2021). Water pre-filtration methods to improve environmental DNA detection by real-time PCR and metabarcoding. PLoS ONE, 16(5), e0250162.

Takeshita, D., Terui, S., Ikeda, K., Mitsuzuka, T., Osathanunkul, M., & Minamoto, T. (2020). Projection range of eDNA analysis in marshes: a suggestion from the Siberian salamander (*Salamandrella keyserlingii*) inhabiting the Kushiro marsh, Japan. PeerJ, 8, e9764.

Takeuchi, A., Iijima, T., Kakuzen, W., Watanabe, S., Yamada, Y., Okamura, A., ... & Tsukamoto, K. (2019). Release of eDNA by different life history stages and during spawning activities of laboratory-reared Japanese eels for interpretation of oceanic survey data. Scientific Reports, 9, 6074.

Turner, C. R., Barnes, M. A., Xu, C. C., Jones, S. E., Jerde, C. L., & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous macrobial eDNA. Methods in Ecology and Evolution, 5(7), 676-684.

Uchii, K., Doi, H., Okahashi, T., Katano, I., Yamanaka, H., Sakata, M. K., & Minamoto, T. (2019). Comparison of inhibition resistance among PCR reagents for detection and quantification of environmental DNA. Environmental DNA, 1(4), 359-367.

Wang, L. D., Chen, H. G., Guo, J. G., Zeng, X. J., Hong, X. L., Xiong, J. J., ... & Zhou, X. N. (2009). A strategy to control transmission of *Schistosoma japonicum* in China. New England Journal of Medicine, 360(2), 121-128.

World Health Organization. (2023). Home/Newsroom/Fact sheets/Detail/Schistosomiasis. https://www.who.int/news-room/fact-sheets/detail/schistosomiasis (Accessed on December 26, 2023)

Xu, Y. Z., & Dresden, M. H. (1989). *Schistosoma mansoni*: egg morphology and hatchability. The Journal of Parasitology, 75(3), 481-483.

Yates, M. C., Fraser, D. J., & Derry, A. M. (2019). Meta-analysis supports further refinement of eDNA for monitoring aquatic species-specific abundance in nature. Environmental DNA, 1(1), 5-13.

Tables

Table 1. Summary of *S. mansoni* eDNA concentrations (copies per mL water) and proportions for each size fraction and treatment (except for uninfected tanks) in the laboratory experiment.

Treatment	Size fraction [µm]	eDNA concentration		eDNA proportion	
		Mean	SD	Mean	SD
(i) Eggs	0.4–0.8	46.8	22.8	0.0%	0.0%
(i) Eggs	0.8–3	804.0	519.0	0.7%	0.6%
(i) Eggs	3–10	1202.7	294.6	0.9%	0.5%
(i) Eggs	>10	145599.7	61414.8	98.3%	1.0%
(ii) Miracidia	0.4–0.8	9.2	7.1	0.0%	0.0%
(ii) Miracidia	0.8–3	181.4	86.9	0.2%	0.1%
(ii) Miracidia	3–10	248.7	147.0	0.2%	0.2%
(ii) Miracidia	>10	120600.3	30017.0	99.6%	0.3%
(iii) 2-wpi	0.4–0.8	0.4	0.5	11.8%	16.5%
(iii) 2-wpi	0.8–3	1.8	2.3	29.0%	34.1%
(iii) 2-wpi	3–10	2.8	4.4	30.0%	11.5%
(iii) 2-wpi	>10	3.0	4.7	29.2%	11.2%
(iv) 3-wpi	0.4–0.8	173.6	228.9	1.3%	1.6%
(iv) 3-wpi	0.8–3	1063.9	1220.1	8.0%	8.6%
(iv) 3-wpi	3–10	2178.3	2611.4	16.9%	18.2%
(iv) 3-wpi	>10	9681.9	5667.5	73.8%	28.2%
(v) 4-wpi	0.4–0.8	89.5	79.6	1.1%	0.6%
(v) 4-wpi	0.8–3	1230.0	571.4	15.5%	5.2%
(v) 4-wpi	3–10	1236.8	628.7	16.2%	8.7%
(v) 4-wpi	>10	5689.1	2802.3	67.2%	14.2%
(vi) 6-wpi	0.4–0.8	54.0	82.7	0.2%	0.1%
(vi) 6-wpi	0.8–3	476.5	269.5	3.1%	2.5%
(vi) 6-wpi	3–10	970.3	359.3	6.3%	3.8%
(vi) 6-wpi	>10	19902.4	18137.5	90.4%	6.2%

Note: SD, standard deviation.

Table 2. Summary of the SODM for *S. mansoni* eDNA detection with the posterior medians and 95 % Cis of each parameter (upper) and the estimated probabilities at three hierarchical levels (Ψ, θ, and ρ) in each survey site (lower).

Model formula	Parameter	Median (95% CI)
Ψ(Infection rate) θ(Volume) ρ(EC)	β(Intercept)	–0.888 (–1.823, –0.081)
	β(Infection rate)	0.475 (–0.458, 1.573)
	α(Intercept)	0.909 (–0.182, 2.002)
	α(Volume)	0.582 (–0.105, 1.243)
	δ(Intercept)	1.620 (1.068, 2.272)
	δ(EC)	-0.603 (–1.164, –0.082)

Site	Ψ			θ			ρ		
	Median	2.5%	97.5%	Median	2.5%	97.5%	Median	2.5%	97.5%
GA1	0.246	0.055	0.550	0.595	0.420	0.752	0.939	0.850	0.984
GA4	0.275	0.060	0.607	0.391	0.220	0.597	0.747	0.517	0.893
RA4	0.186	0.034	0.466	0.888	0.408	0.996	0.936	0.848	0.983
RA6	0.289	0.060	0.628	0.924	0.385	0.999	0.974	0.875	0.998
RB3	0.333	0.056	0.725	0.911	0.391	0.998	0.968	0.871	0.996
GA3	0.384	0.050	0.833	0.602	0.423	0.763	0.610	0.266	0.868
RA_public	0.186	0.034	0.466	0.791	0.432	0.964	0.978	0.880	0.998
site1	0.168	0.024	0.456	0.839	0.423	0.985	0.976	0.876	0.998
site2	0.025	0.000	0.670	0.926	0.385	0.999	0.977	0.878	0.998
site3	0.086	0.001	0.492	0.900	0.400	0.997	0.978	0.880	0.999
site4	0.148	0.014	0.456	0.900	0.400	0.997	0.978	0.880	0.998

Note: parameters β, α, and δ represent the Bayesian estimates of site-level probability Ψ, sample-level probability θ, and PCR replicate-level probability ρ.

Figure legends

Figure 1. Survey sites in Lake Victoria. Maps were created using QGIS (version 2.14.7-Essen) based on the dataset from Natural Earth (https://www.naturalearthdata.com).

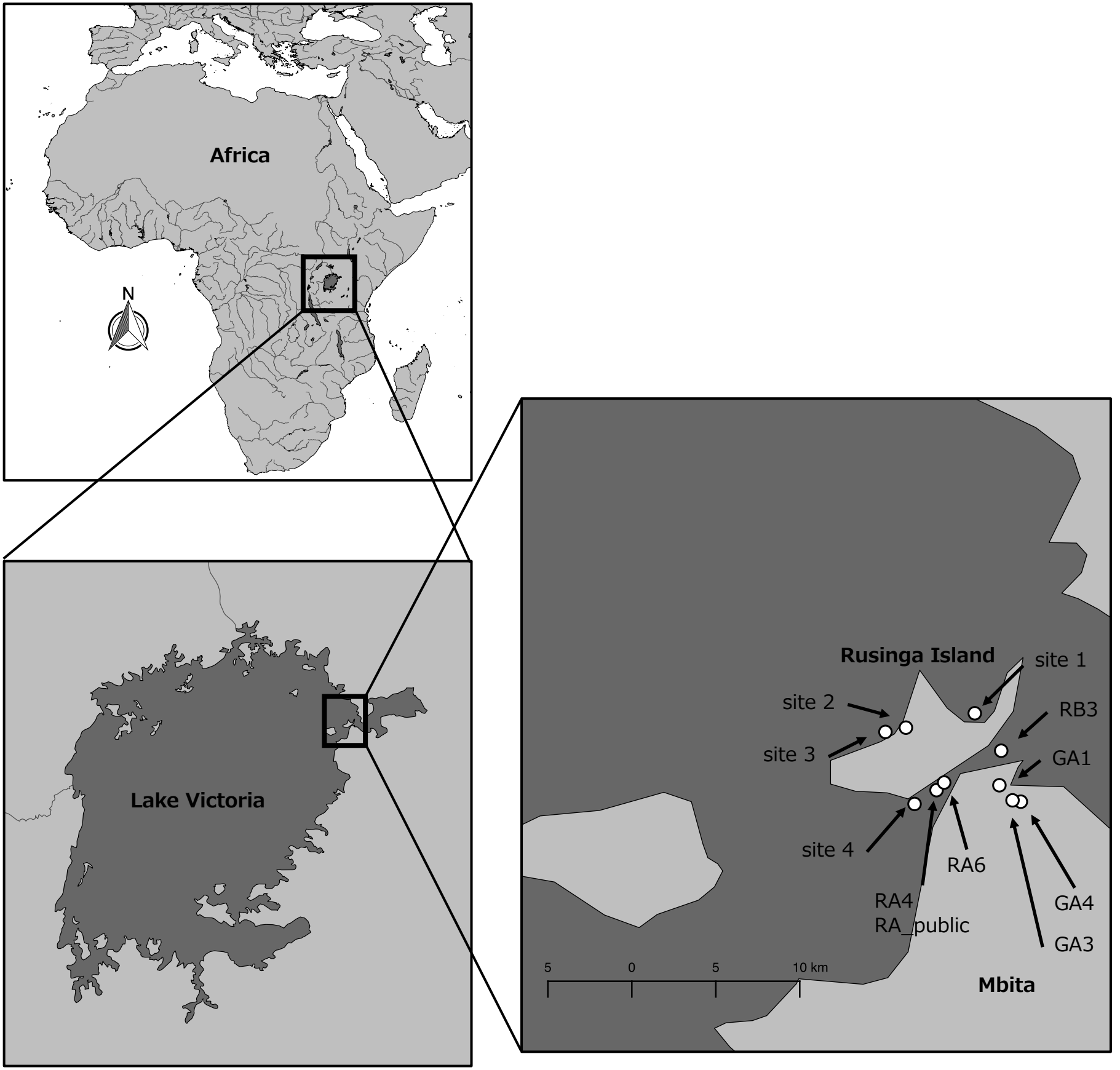
Figure 2. Comparison of *S. mansoni* eDNA concentrations in different size fractions for each treatment (except for uninfected tanks). Factors with different letters are significantly different ($P < 0.05$) based on post-hoc multiple comparison tests. n.s., not significant ($P > 0.05$).

Figure 3. Time-series changes in *S. mansoni* eDNA concentrations (upper boxes) and proportions (lower boxes) after infection of intermediate snails at each size fraction. Asterisks and dots indicate the significant (***$P < 0.001$, **$P < 0.01$, and *$P < 0.05$) and marginal ($P < 0.1$) differences between the factors.

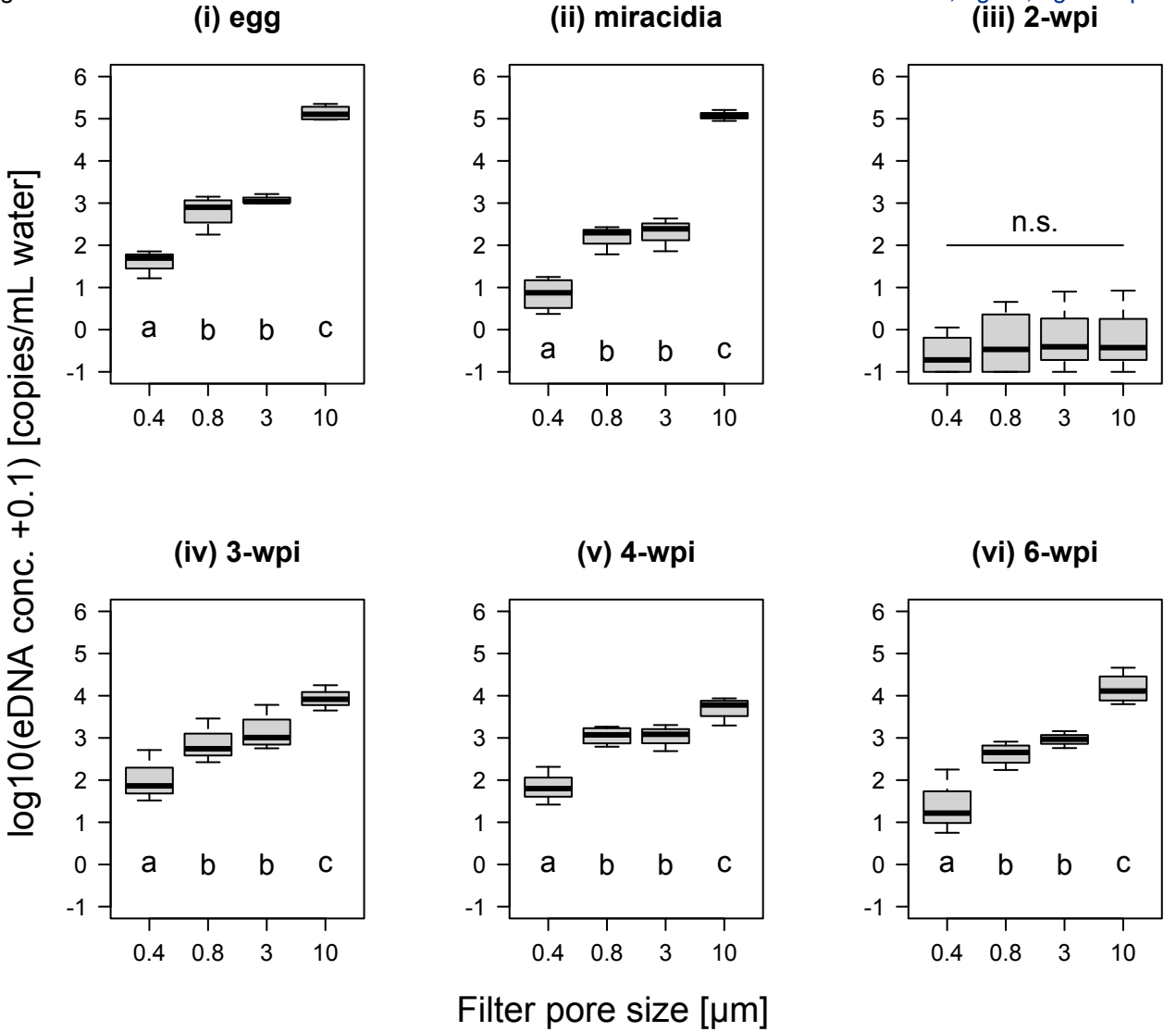
Figure 4. Estimated probabilities at each hierarchical level based on the SODM. The figure shows the relationships between infection rate and site-level occurrence probability Ψ (left), between filtration volume and sample-level occurrence probability θ (middle), and between conductivity and PCR replicate-level detection probability ρ (right). Symbols indicate the estimates of posterior medians with 95 % CIs at each survey site.

Figure

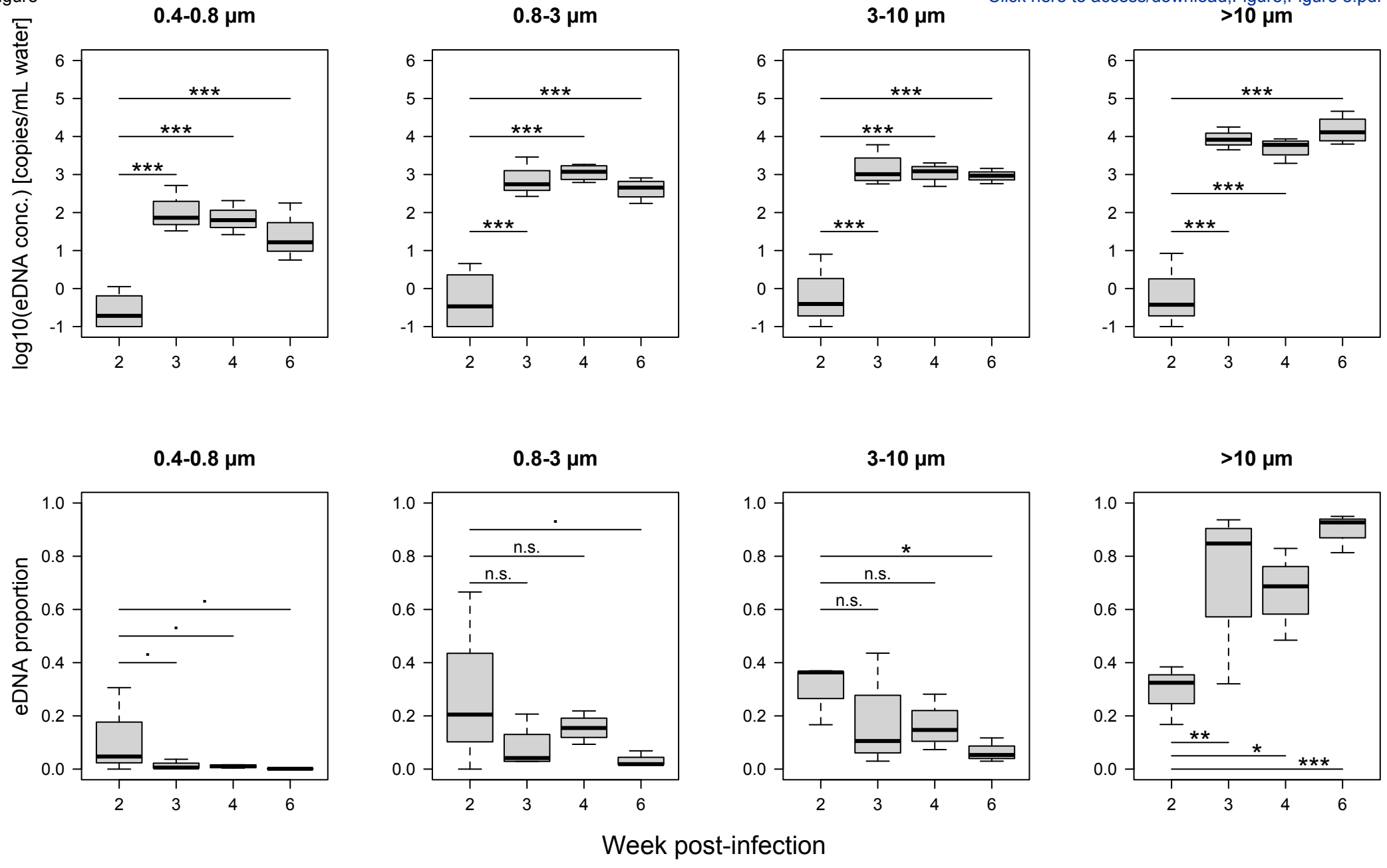
Click here to access/download;Figure;Figure 1.pdf



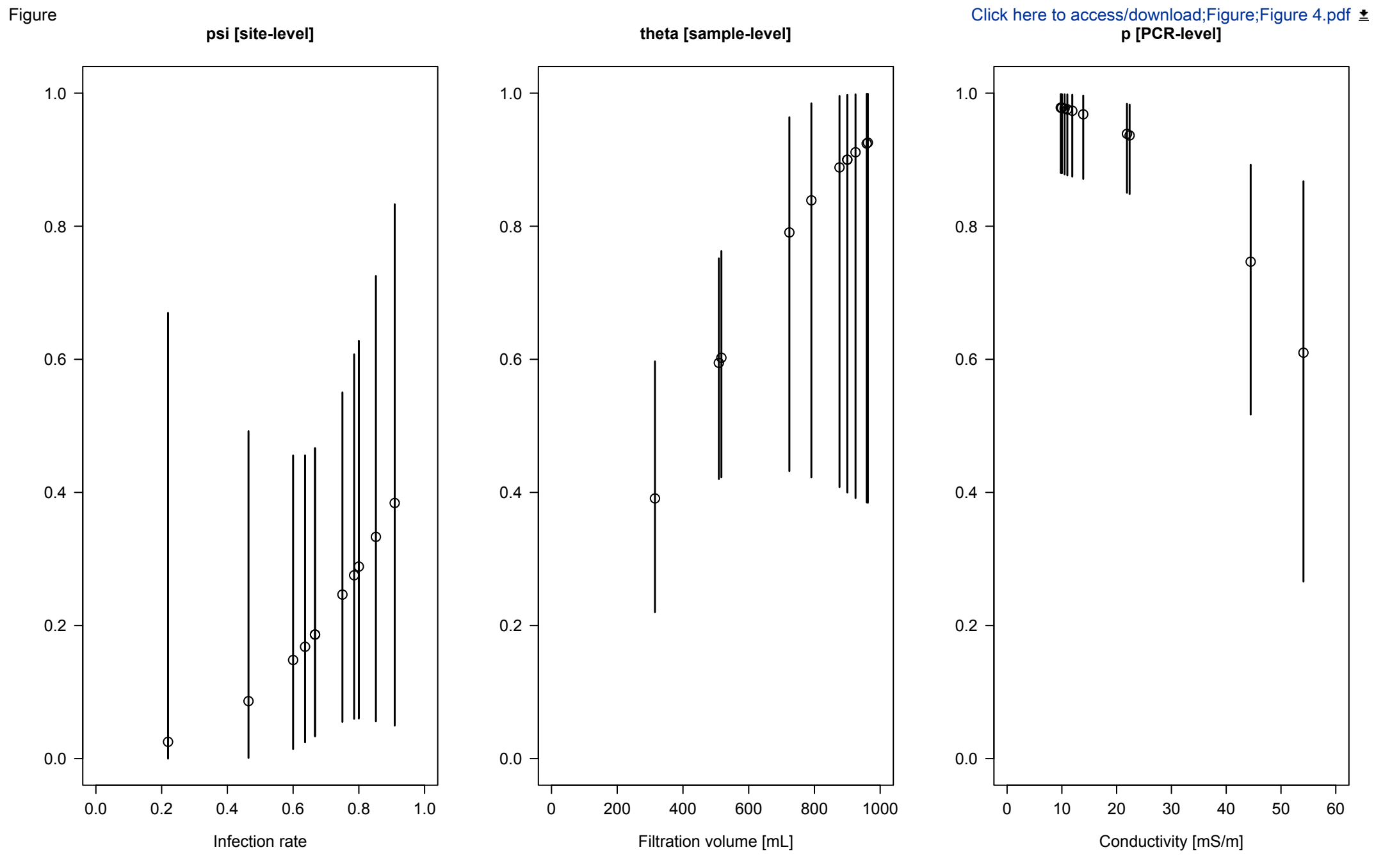
Figure



Figure



Figure

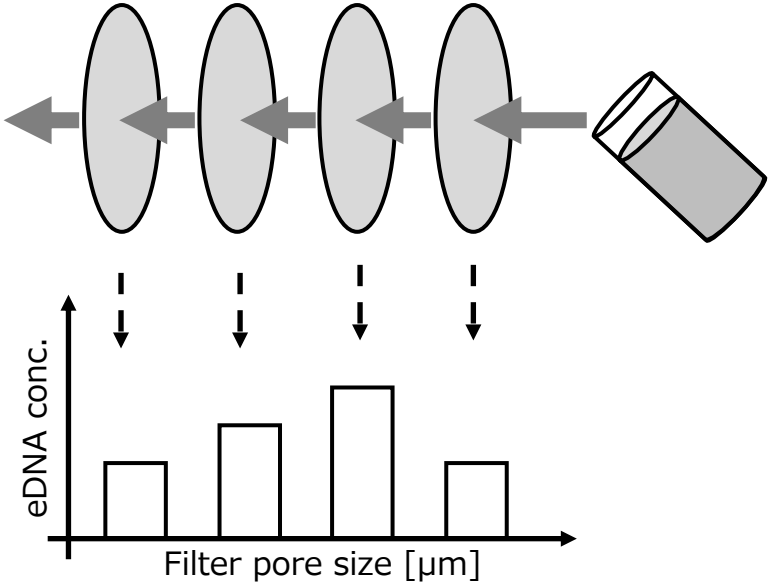


Graphical Abstract

Fine-tuning of *S. mansoni* eDNA collection and detection

Laboratory experiments

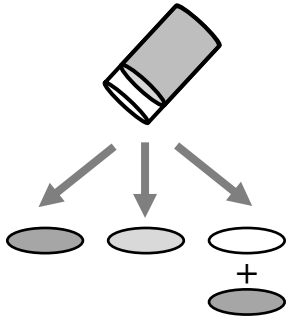
Sequential filtration



Quantification of size-fractioned eDNA

Field survey

1st



Filtration method choice

2nd

Site occupancy-detection modeling

eDNA detection → Occupancy/detection probability

Environmental parameter