THE EFFECT OF PESTICIDE POLLUTANTS ON TRANSMISSION OF SCHISTOSOMIASIS IN WESTERN KENYA

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted in part or whole for an award in any institution.

Signature:  Date: July 31st, 2023

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Recommendation

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DEDICATION

I dedicate this to the Richard Thaler,

Who first introduced the concept of the sunk-cost fallacy.

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ABSTRACT

Schistosomiasis is caused by trematodes that utilise planorbid snails as their intermediate hosts in Africa. The study aimed to determine the effect of pesticides on the transmission of the disease. This was done by first investigating the effect of two commonly used pesticides, imidacloprid, a neonicotinoid, and diazinon, an organophosphate, on the host snails, *Biomphalaria* and *Bulinus*, to determine their tolerance. The tolerance of the snail's competitors and predators was also investigated. Using laboratory experiments, sensitivity distribution graphs were used to determine that the host snails were the most tolerant taxa to the pesticides chosen. Data from the field showed that the snails were present in moderately polluted sites or greater, with a distinct lack of presence in pristine waters. Binomial and mixed effects general linear models were used to determine a minimum effects model of variables that show the distribution of host snails, and were put into a principal component analysis which showed an antagonistic relationship between pesticide pollution and competitors of host snails. This led the study to conclude that pesticide pollution in the field increases the abundance of host snails by reducing the number of competitors, due to the competitor's higher sensitivity to the pesticides. The effect of pesticides on the parasite itself were then investigated by studying the effects of the same two pesticides on the free-living life-stages of *Schistosoma mansoni*. The dose-response calculations revealed the tolerance to be 50-60 times the concentration of what was found in the field. Miracidia were further exposed to sub-lethal doses at 20% the EC50s, where it was shown that these concentrations could reduce the proportion of infected snails. However, these concentrations were 500-600 times greater than those in the field. Therefore, the study here shows that pesticide pollution has the potential to increase snail hosts dominance over their competitors in polluted environments, while not affecting the parasite at environmentally relevant concentrations, leading to an overall increase in risk of disease transmission. As pesticide pollution could increase the risk of schistosomiasis, it must be monitored within habitats to determine the change in risk across seasons. The study evaluated the potential of the SPEARpesticide bioindicator for use in Kenya and found it to be promising. Thus, the government of Kenya could potentially use the bioindicator to cheaply and quickly assess pesticide pollution in the rivers of Kenya and use the information to map out potential areas where the risk of schistosomiasis could increase in the coming years.

CONTENTS

LIST OF ABBREVIATIONS AND ACRONYMS

BMWP	Biological Monitoring Working Party index
CDC	Centre for Disease Control
EC50	Effective concentration at which 50% of organisms are immobilized in 24hrs
EEC	Estimated Environmental Concentrations
EPT	Ephemeroptera, Plecoptera and Trichoptera
FGS	Female Genital Schistosomiasis
GP	General Practitioner/ Doctor
LC50	Lethal concentration at which 50% of organisms die in 24hrs
MDA	Mass Drug Administration
PZQ	Praziquantel
SASS5	South African Scoring System 5
SPEAR	Species At Risk
$SPEAR_{pesticides}$	Species at Risk index for pesticides
SSD	Species Sensitivity Distribution
TU	Toxic Unit
WHO	World Health Organisation

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Human Schistosomiasis is a disease caused by blood-flukes (*Schistosoma mansoni, S. haematobium* and *S. japonica*). The schistosome parasites rely on two hosts (a vertebrate host: mammals and invertebrate: snails) to complete their life cycle (CDC, 2018). The burden of the disease is therefore focused in areas where these hosts live in sympatry, allowing the infectious trematode easy transmission between hosts. The human hosts, and a snail, from the genus *Biomphalaria*, *Bulinus* and *Onchomelania* depending on the species of *Schistosoma* is where the trematode develops. The flukes develop within the hosts, utilising the host nutrients for their own reproduction and dispersal (WHO, 2017). The snail, which is an obligate intermediate host is invaded first, during which the trematode undergoes several rounds of asexual reproduction to produce hundreds of human-infective cercariae into the water bodies the snails reside in (CDC, 2018). This is the point at which the schistosomes can gain entry to humans and subsequently cause the disease.

The transmission into the humans is thus restricted to the habitats of the snails. The snail stage of the schistosomes has been suggested as a possible target for control, since eliminating either the helminths within the snails or the snails entirely, automatically reduces the number of infectious cercariae, and thereby decreasing the disease transmission and burden (King, 2015; Sokolow *et al.*, 2016). However, such strategies may evoke ecological food-chain collapse, since snails are vital to the freshwater bodies they reside in as a source of food for almost all classes of Animalia (Brown, 2002). Thus, the biology of the host snails and their interactions in their ecosystems must be fully understood before such measures are undertaken. However, as these solutions are being investigated, it is also imperative to characterise the habitats to understand future trends of schistosomiasis transmission. As the human population increases, their activities alter the communities of the environment they encroach into. For example, near Lake Malawi, settlements led to overfishing, which released the local snail populations from predation pressure, increasing their numbers and subsequently, increase in schistosomiasis cases (Madsen, 2001). Similarly, farming practices could affect current trends of schistosomiasis, considering the agrochemicals used to improve crop yields and vector control have been linked to loss of biodiversity in nearby streams (Beketov, 2013).

For snails to survive and to proliferate, environmental conditions must be conducive for the respective species. The ecology of the host snails has not been extensively studied, especially in freshwater streams and water shed areas in sub-Saharan Africa. The few studies that have been done suggest that water temperature, water fluctuation, vegetation cover, snail density and water quality might impact on /snail occurrence and density (Perez-Saez *et al.*, 2016). The snails have previously been linked to moderately polluted waters, due to the preferential conditions that eutrophication causes. Further studies found that molluscs are relatively less sensitive to organic and metal pollutants than other taxa (Johnson, 2007; WHO, 2000; Wogram & Liess, 2001). This information raises questions about the ecological consequences of the vast amounts of pollutants that find their way into water bodies, and their effect on the schistosome host snails and cohabiting macroinvertebrates, some of which naturally predate on snails or compete with them for resources. Snails, their predators, and competitors might be differentially sensitive to certain environmental factors including pollutants that might affect the ecological balance and consequently, the potential for disease transmission (Perez-Saez *et al.*, 2016; Wogram & Liess, 2001). Furthermore, the effect of such pollutants on the parasite, could be too detrimental and therefore would nullify the risk of transmission no matter the increase in hosts.

1.2 Statement of the problem

Schistosomiasis is a common parasitic disease in western Kenya and is a serious burden to growing children. The area formerly known as Nyanza Province, is also known for its fertile lands that are used by locals for subsistence farming as well as large scale commercial farming, that require the use of agrochemicals. As such, there is potential for pesticide pollution into the surface waters of the areas, where the snail-hosts reside, and transmission occurs. Therefore, there is an urgent need to evaluate the effects of the commonly used pesticide chemicals on the aquatic macro-invertebrate community as there is potential for these chemicals to alter the community composition to favour the host snails. Furthermore, the effect of the chemicals on parasite itself has not been studied. Therefore, the overall change in transmission trends cannot be predicted until the effects are elucidated.

1.3 Objectives

1.3.1 General objective

To investigate the effect of commonly used pesticide pollution on the transmission of schistosomiasis in Western Kenya.

1.3.2 Specific objectives

i. To determine the distribution of schistosome-host snails and associated macro-invertebrates in the inland water bodies around Lake Victoria, western Kenya.

ii. To determine the effect of pesticides on snail and macroinvertebrate communities in water bodies around L. Victoria, western Kenya.

iii. To determine the infectivity of *Schistosoma* on snails in the presence of pesticides commonly found in the water bodies around L. Victoria, western Kenya.

1.4 Hypotheses

i. *Schistosoma*-host snails are not more susceptible to pesticide pollutants than their sympatric predators or competitors.

ii. There is no significant association between biotic and abiotic environmental conditions and population distribution of *Schistosoma*-host snails in western Kenya.

iii. *Schistosoma mansoni* and *Schistosoma haematobium* are not compromised in their infectivity and maturation due to pesticide contaminants.

1.5 Justification

Schistosomiasis causes more than 200,000 world-wide fatalities a year. In western Kenya, the disease is caused by two species of the parasite, *Schistosoma mansoni* and *Schistosoma haematobium* which are hosted by the planorbid snails of the genus *Bulinus and Biomphalaria,* respectively. The ecology of these hosts has not been extensively studied in the area, and there is limited data on transmission along the freshwater streams and watershed areas of sub-Saharan Africa, (SSA). This study aims at filling some of these knowledge gaps, as well as to address the trend of increased agrochemical use in the area and its effects on the disease transmission trends or control programmes for schistosomiasis.

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

2.1.1 Schistosomiasis

Schistosomiasis is a disease caused by a parasitic helminthes from the genus *Schistosoma*. The genus contains six species groups, which are: *Schistosoma mansoni, S. haematobium, S. japonicum, S. mekongi, S. intercalatum* and *S. guinuensis*. Their distribution is heavily dependent on their hosts. They live the life of typical trematodes, wherein they rely on several hosts to complete their lifecycles: a definitive mammalian host and an intermediate host, the snail (Cribb, 2001). The parasites survive by residing in their definitive host's circulatory system and redirecting the host's nutrients to mature and develop their own progeny. Specifically, the adult worms digest erythrocytes and obtain their energy through metabolism of the glucose they ingest (Barrett, 2009; van Oordt, 1985). They utilise the energy and nutrients from the erythrocytes to produce their own eggs, which they release with their hosts excrement, with the intention that they will reach a body of freshwater. In freshwater, the eggs are able to hatch into miracidia that seek out a snail host to infect where a single miracidium can multiply asexually into thousands of sporocysts that mature into human-infecting cercariae (Colley *et al.*, 2014). These cercariae are released by the snails daily in the hundreds. They seek out humans in the water whom they can infect by penetrating through the skin, transforming into schistosomules. This stage traverses the human's blood circulatory system before reaching the liver where they remain until they mature. Once mature, the *Schistosoma* then move to either the bladder or intestines, depending on the species, where male and female worms form a mating pair and live *in copula* for the rest of their lives, releasing eggs with the host's excrement and beginning the life cycle over again (Fig 2.1) (Colley *et al.*, 2014; Gryseels *et al.*, 2006; King *et al.*, 2009). The disease is therefore transmitted indirectly between the two host species, though, certain conditions increase likelihood of transmission. These include low socioeconomic health of the people, where water supply and wastewater facilities are inadequate and human excrement is able to find its way back into the freshwaters used for domestic purposes (Grimes *et al.*, 2015). This increases the risk as people are forced to expose themselves multiple times as well as the higher risk due to more excrement in the water, which is the source of eggs. The presence of snails is of course a necessity for transmission to occur, therefore conditions that affect their presence are also highly relevant. As such the study designed below aims to elucidate these conditions.

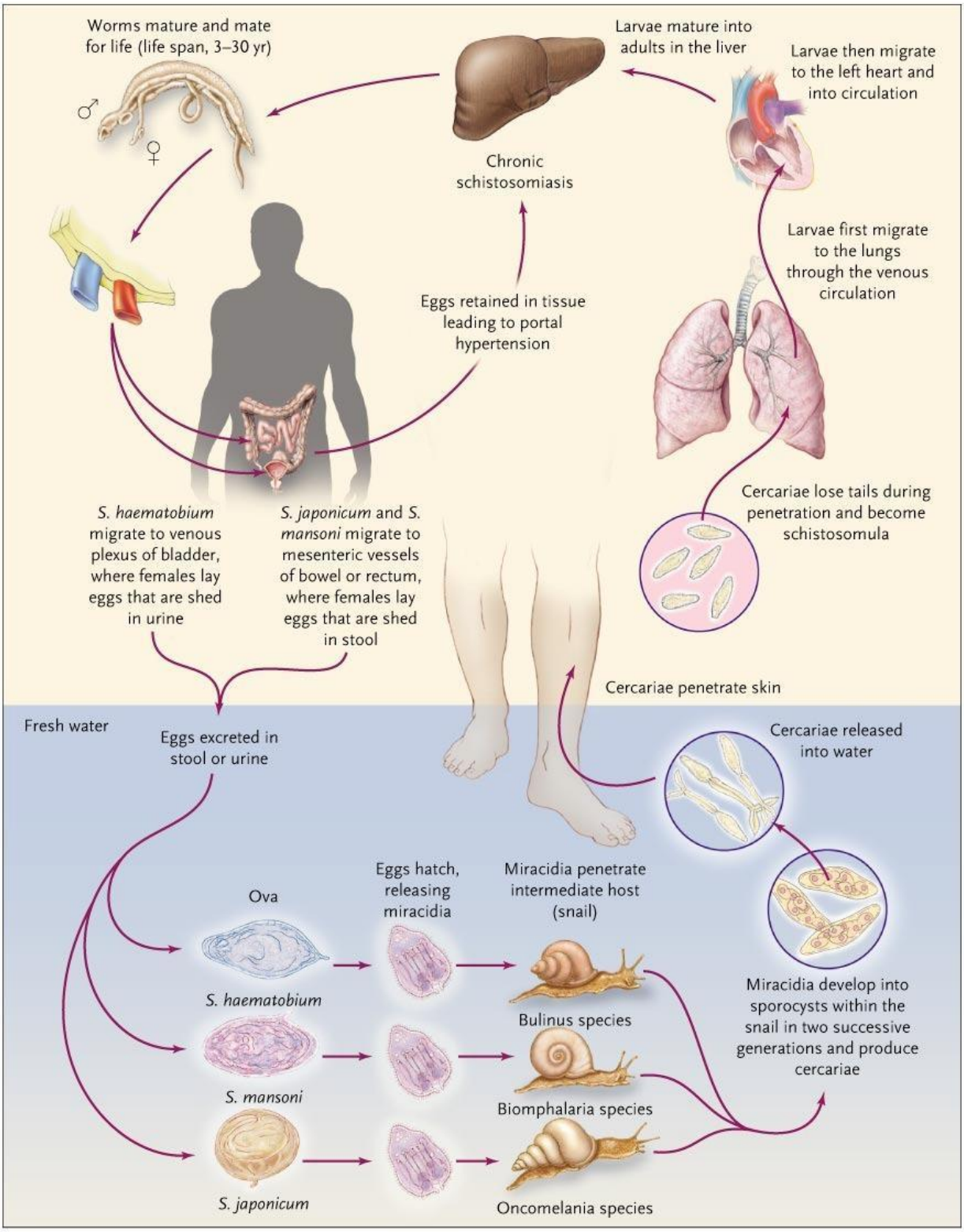


Figure 2.1. The life cycle of the various *Schistosoma* species showing the host species choice of each *Schistosoma* as well as the route within the human body to reach their desired location in the human body (King, 2009).

2.1.2 Distribution

Schistosomiasis transmission occurs in areas of South America, the Caribbean, the Middle East, Cambodia and Laos, Corsica, China and Southeast Asia, and in Africa, in small water bodies, lakes or rivers of sub-Saharan Africa, along the Nile River in Sudan and Egypt and parts of west Africa (CDC, 2018). However, it is known that majority of cases – up to 90% - occur in Africa (WHO, 2022). Across all these regions, transmission is facilitated by lack of access to clean water and toilets and the presence of snail hosts for various *Schistosoma* species to infect. In Africa, two species of *Schistosoma* occur, *S. mansoni* and *S. haematobium*, which utilise *Biomphalaria spp* and *Bulinus africanus spp* snails as hosts, respectively (Brown, 2002). In Kenya, both species present in Africa occur, with *S. mansoni* utilising the various *Biomphalaria* species present such as *B. pfeifferi, B. choanphalla* and *B. sudanica*; while *S. haematobium* have variety of Buliniid hosts such as *B. africanus s.s., B. globusus* and *B. tropicus* (Handzel *et al.*, 2003) (Fig 2.2).

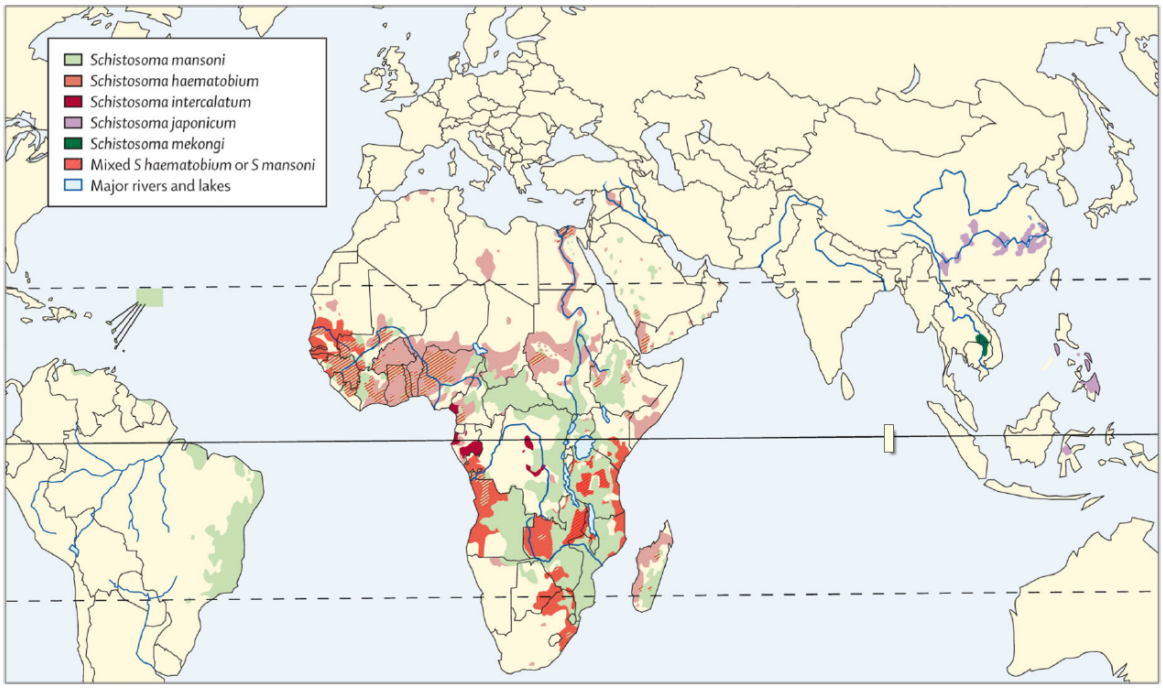


Figure 2.2: Distribution of the different species of *Schistosoma* (King, 2015).

Transmission zones need to be continuously monitored for developments, and the factors that could affect transmission need to be assessed to predict future hotspots and where to concentrate control strategies. Such factors are those that affect snail presence, human presence and activity or those that affect the parasite. Such factors include climate change (Fig 2.3) but should also be extended to ecologically disruptive activities such as the uncontrolled use of pesticides.

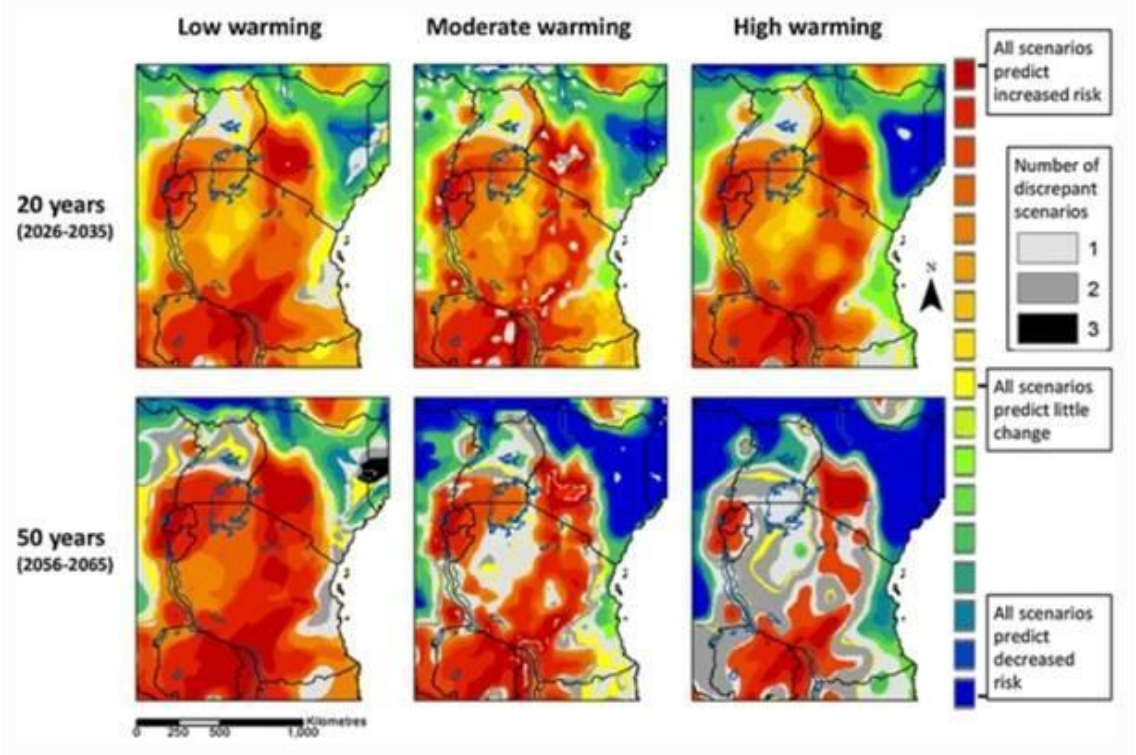


Figure 2.3: The predicted change in schistosomiasis risk in the upcoming decades under various warming scenarios according to models (Mccreesh *et al.*, 2015).

2.1.3 Pathogenesis

The pathology of the disease can range from mild, - in which case patients may be asymptomatic and not even realise they have the parasite (CDC, 2018) to severe – in which case the heavy burden of infections can lead to disfiguring, disabling and life-threatening disease (Carod-Artal, 2012). Travellers or immigrants not exposed to *Schistosoma* antigens at an early age react more severely to exposure to these antigens, manifesting in what is referred to as acute schistosomiasis; which in early days was known as Katayama fever (Doherty *et al.*, 1996). The overall manifestation may seem sudden, due to the prolonged prepatent period over which patients may not connect exposure to cercariae-infested water to their present symptoms (Ross *et al.*, 2007) The symptoms may include but not limited to fever, fatigue, malaise, myalgia and abdominal pains (Gryseels *et al.*, 2006; Rocha *et al.*, 1995).

Longer infections may lead to manifestations of more severe symptoms, which are then diagnosed as “chronic schistosomiasis”. The chronic phases of the disease are caused by the accumulation of eggs from mated adult couples within the host (Burke *et al.*, 2009). These

eggs do not get passed out of the body as is optimal and instead traverse the hosts body, getting stuck in the intestines and/or liver or in the bladder or urogenital tract, depending on the *Schistosoma*-species, (King 2015). In *S. mansoni, S. japonicum and S. mekongi*, the disease manifests as intestinal schistosomiasis characterised by diarrhoea, bleeding from the rectum and intermittent abdominal pains. Some victims may be unable to regulate their immune response to parasite egg antigens, and therefore suffer extensive fibrosis, causing hepatosplenic disease with periportal fibrosis (Cheever, 1968; Colley, 1986). The progression from invasion to advanced fibrosis can take between 5 and 15 years (Gryseels, 1992); however, periportal fibrosis has been recorded in children as young as 6 years old (Doehring-Schwerdtfeger, 1990).

On the other hand, *S. haematobium* infection manifests as urinary schistosomiasis, characterised mainly by haematuria, but accompanying other symptoms such as burning micturition, suprapubic discomfort and urinary frequency (Gryseels *et al.*, 2006). *Schisotosoma haematobium* infections are also implicated in other health risks. Their presence is strongly associated with squamous-cell carcinomas of the bladder (Schwartz, 1981) and female genital schistosomiasis (FGS), which severely impacts women's reproductive health (Kjetland *et al.*, 2012). The damage to the genital tracts in such severe cases are not repairable, which adds complications to the cases as evidence mounts that the lesions caused can increase risk of HIV transmission (Kjetland *et al.*, 2006). Urogenital schistosomiasis can lead to inflammation of the prostate and/or testicles, haematospermia, lowered fertility and even pain during intercourse in men (Gryseels *et al.*, 2006). However, these are more susceptible to treatment than FGS (Leutscher, 2000, 2009). Amongst the symptoms, *Schistosoma*-trematodes also cause other non-specific ailments such as anaemia, malnutrition and, most devastatingly: impaired development in children (King & Dangerfield-Cha 2008). Studies have shown these to be caused by continued inflammation which affects normal iron metabolism and cognitive functioning (Bustinduy *et al.*, 2011; Ezeamama *et al.*, 2005; Friedman *et al.*, 2005). There is also the unlikely manifestation which is caused by the accumulation of *Schistosoma* eggs in the brain of the host, causing cerebral schistosomiasis or neuroschistosomiasis (Carod-Artal, 2008; Carod-Artal, 2012; Ross, 2012). Such cases are also under-recognized, and the true burden is hardly realised. In Malawi, half of patients' non-traumatic spinal cord injuries were caused by schistosomiasis (Naus, 2003). Data from Chinese hospitals estimate 4% of *S. japonicum* cases develop neuro-schistosomiasis (Chen & Mott, 1988).

2.1.4 Diagnosis

Diagnosis of schistosomiasis is reliant on the oldest and most cost-effective method: the Kato-Katz Technique (Feldmeier & Poggensee, 1993). This entails microscopic examination of faecal samples from the patient for the characteristic *Schistosoma* eggs (Fig 2.4). The technique remains virtually unchanged since its inception in 1972, and requires a stool sample from the patient to be sieved through a wire mesh or $1mm^2$ (Katz *et al.,* 1972). The sieved stool should then be placed onto a microscope slide and covered with a cellophane coated with Malachite Green dye. The slide should then be pressed to spread the sample evenly across the slide and can then be examined for eggs (WHO, 2019). The downside of this diagnostic tool is that oviposition occurs approximately 6 weeks post-cercarial infection, and so early diagnosis is not possible, during which time serious symptoms can manifest (Wang *et al.*, 2011)

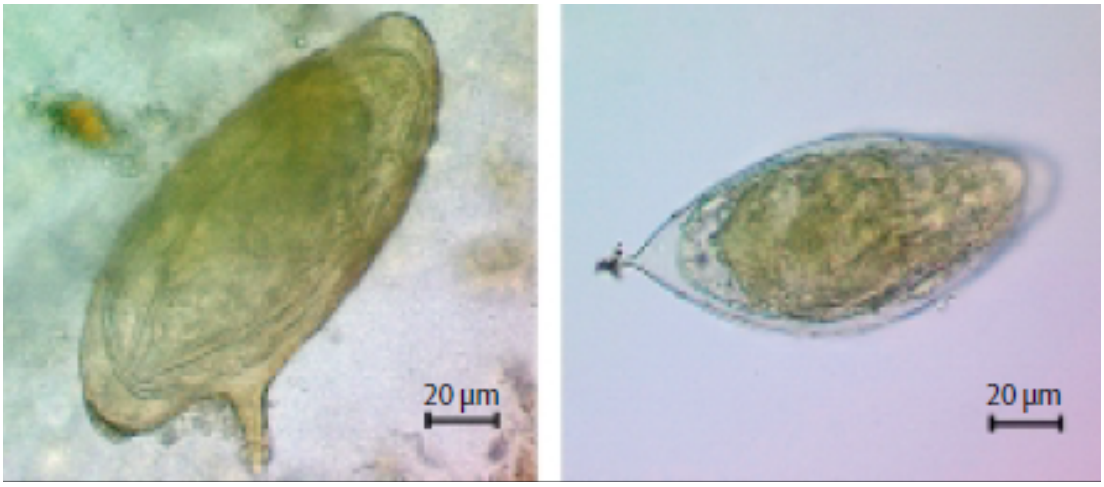


Figure 2.4 *Schistosoma mansoni* egg on the left characterised by a lateral spine and *Schistosoma haematobium* egg on the right characterised terminal spine. Adapted from (Ross *et al.*, 2007).

For travellers, diagnosis is assisted with other tools such as medical history in which a patient's recent travels are noted, particularly those travelling from endemic regions and having been in contact with freshwater during travels (such as lakes or rivers). Physical examinations may also assist, as urticarial rash where cercariae penetrated may be present, or palpation of the abdomen, hepatomegaly or splenomegaly. Other laboratory investigations such as full blood count may be taken, resulting in eosinophilia for acute infections or anaemia and thrombocytopenia for chronic schistosomiasis. Additionally, radiology can assist

in diagnosing the particular morbidities of each case, such as chest radiographs (Gray *et al.*, 2011). Abdominal ultrasound can be used to establish extent of liver and spleen pathology while pelvic ultrasound to determine extent of bladder, uretal and renal pathology (Ross *et al.*, 2002).

New diagnostic tools have since been developed such antigen tests for serological detection of proteoglycans regurgitated by the worms (Deelder *et al.*, 1980). These proteoglycans are the negatively charged circulating anodic antigen (CAA) and positively charged circulating cathodic antigen (CCA) which can be detected in serum or urine by enzyme-linked immunosorbent assay (ELISA) (van Dam *et al.*, 2013). Their presence is considered indicative of active infection with live worm and can also be detected before the worms start producing eggs (Van Dam *et al.*, 1996), which allows for early detection of infection (McManus *et al.*, 2018). As the levels of antigens correlate with the intensity of infection (Van Lieshout *et al.*, 1995), the assays prove valuable in research of epidemiology in response to treatment (McManus *et al.*, 2018). However, the assays may not be suited for diagnosis in areas with low endemicity or in travellers who are likely to only have a few worms (Van Lieshout *et al.*, 1997). Another tool used is PCR to detect the DNA of schistosomes in urine (Obeng *et al.*, 2008) or stool (Meurs, 2015). The tool has the advantage of being more sensitive than other methods (He *et al.*, 2018). However, it is expensive and therefore has found its niche in high-resource settings (McManus *et al.*, 2018).

2.1.5 Treatment

The treatment of schistosomiasis requires a standard dose of 40mg/kg of Praziquantel (WHO, 2002), which can eliminate the presence of eggs in 70-100% of cases, or reduce egg-count and antigen concentrations by more than 95% (Reich, 1998), although higher doses of 60mg/kg are recommended for *S. japonicum* and *S. mekongi* infections (King 2015). Effective praziquantel dosage regimen of 60 mg/kg orally in divided doses, however, a single 40 mg/kg dose is administered with 66-95% efficacy for epidemiological studies and population-based preventive chemotherapy control programmes (Gryseels *et al.*, 2006). Efficacy of 95-100% can be achieved with re-treatment four to six weeks after the first dose (Li *et al.*, 2000).

Praziquantel has very few side effects, such as transient nausea, rash, dizziness, and pruritus (Cioli & Pica-Mattoccia, 2003; Doenhoff *et al.*, 2008). Care must be taken for patients with

concurrent *Taenia solium* cysticercosis, as the death of *Taenia* parasites in the ocular region due to praziquantel can cause irreparable eye lesions (Flisser *et al.*, 1993; Johnson, 1986; Torres & Jaime, 1989). The intense inflammatory reactions initiated from dying *Taenia* cysts could also induce seizures or cerebral infarctions (Garcia *et al.*, 2020).

Resistance to praziquantel is a looming concern fuelled by the reports of *S. mansoni* and *S. haematobium* infections non-responsive to treatment in some areas (Fong & Cheung, 1997; Ismail *et al.*, 1999). Evidence suggests that drug-tolerant strains of *Schistosoma* may have altered tegumental architecture (Gray *et al.*, 2011). However, many communities have undergone treatment with praziquantel for over a decade without loss of efficacy (Wang *et al.*, 2012). It is hypothesized that, because *Schistosoma* reproduction is sexual and the generation time is long, drug resistance may take many years before it can take hold in the trematode population and become a public health issue (Gray *et al.*, 2011).

Prophylaxis of schistosomiasis is not possible with Praziquantel as it cannot kill the immature schistosomula stage (Gray *et al.*, 2010; Ross *et al.*, 2015) and its short half-life of up to 1.5 hours (Gray *et al.*, 2011). In this regard, artemether has shown high efficacy in its effect against juvenile schistosomes less than 21 days old (Utzinger, 2007). Indeed, it has already been used as a chemoprophylactic in China for people at high risk in endemic areas, such as fishermen (Shu-Hua, 2005). However, its benefit against malaria requires that it not be used in low doses in regions endemic for malaria due to the risk of accidentally selecting for artemether resistant *Plasmodium falciparum* (Shu-Hua, 2005; Utzinger, 2007).

Corticosteroids can be used to treat encephalopathy caused by the egg laying of the schistosomes (Farid *et al.*, 1989; Fowler *et al.*, 1999). They may also be used in conjunction with anticonvulsants as adjuvants to praziquantel in neuroschistosomiasis (Gray *et al.*, 2011). The corticosteroids fight against acute allergic reactions and prevent the effects caused by excessive granulomatous inflammation in the central nervous system while the anticonvulsants treat the seizures. Surgery is saved for those that exhibit signs of medullary compression (Carod-Artal, 2008; Ferrari, 2004). Other available drugs include oxamniquine for *S mansoni* (Araujo *et al.*, 2008) and metrifonate for *S haematobium* (Feldmeier & Doehring, 1987). However, their use was discontinued due to the superiority of praziquantel in efficacy, cost of production and less side effects (McManus *et al.*, 2018).

Many drugs are in development for schistosomiasis such as HMG- CoA (3-hydroxy-3-methylglutaryl-CoA) reductase, which was found to impact egg production and

survival of *S. mansoni* (Rojo-Arreola *et al.*, 2014); while other drugs are discovered by means of repurposing drugs such as the protein kinase inhibitor, Imatinib, commonly used in cancer therapy which was discovered as being able to block the activity of a protein Kinase SmAbl1 (Buro *et al.*, 2014). Beside the above drugs, several targets for drugs have also been elucidated which include prolyl oligopeptidase (Fajtová *et al.*, 2015), thioredoxin- glutathione reductase (Johann *et al.*, 2015), glucose transporters (Cabezas-Cruz *et al.*, 2015), a serotonergic G protein- coupled receptor (GPCR) (Chan *et al.*, 2016), a heat shock 70 kDa protein (HSP70) (Ishida & Jolly, 2016), ion (transient receptor potential (TRP)) channels (Bais & Greenberg, 2016) and acetylcholinesterase (Sundaraneedi *et al.*, 2017).

2.1.6 Control

The main control program outlined by the World Health Organization (WHO) for preventative chemotherapy (PC) relies heavily on mass administration of the drug Praziquantel (WHO, 2002), which is too flawed to be effective at elimination (Keenan *et al.*, 2013). The drugs drawbacks are: i) it is ineffective against immature forms, meaning it needs to be taken in two doses 6 weeks apart to properly cure someone, and ii) it does not prevent reinfection, meaning that after a complete cure penetration by cercariae can occur even within the day of PC (Mbanefo *et al.*, 2014). This renders the exercise redundant and wasteful and such shortcomings prevent the interventions from being successful in the long term. As the situation concerning schistosomiasis transmission depends on availability of the intermediate snail hosts and definitive host, the human blocking transmission will require making the hosts unavailable for the parasite (CDC, 2018).

The current system, which is reliant on mass drug administration (MDA), depends on timely and consistent delivery of the drug Praziquantel to the needed areas as well as the participation of the communities and their faith in the system and the drugs. As governments fail on their part, the community faith also falters and participation in future MDA is then compromised. Another issue with MDA of Praziquantel, as stated before, is that it does not prevent reinfection with cercariae, resulting in quick resurgence of the disease once the MDA is completed (Trippler *et al.*, 2021), and remains high until the next round. Combined with the high likelihood of supply chain management issues in a low income countries (Mondale de la Santé & WHO, 2018), MDA will be unable to reduce infection levels in humans enough to achieve elimination. For there to be a maintainable reduction, the point of infection should

also be addressed. The Global Schistosomiasis Alliance recently called for attention to be brought back towards the development of a schistosomiasis vaccine to be used in complement with MDA and water, sanitation and hygiene (WASH) with a one-health approach (The International Society for Neglected Tropical Diseases, 2021). The development of a vaccine for a disease is one of the most promising steps towards prevention, control, and elimination. As such, many candidates have been pursued as a possible vaccine for schistosomiasis (summarized in Figure 2.5). However, this approach is unlikely to take-off and focus on implementing WASH programs alongside MDA would be more viable. This is because it is thought vaccines would take too long to distribute and the disease can be eliminated before full coverage is achieved. Therefore, elimination can be achieved as has been done in other countries such as Japan, China, Philippines and Egypt without the use of vaccines (Rollinson *et al.*, 2013).

Snail control was also readded to the current strategy recommended by the WHO for control of schistosomiasis in endemic areas (WHA, 2012). Its use had been stopped since 1974 with the advent of praziquantel (King & Bertsch, 2015). However, due to the resilience of the disease in avoiding elimination, it was reconsidered as a complementary control strategy alongside MDA (Sokolow *et al.*, 2016). However, the downstream toxic effects of molluscicides on the environment present a challenge to use in the environmentally conscious world (King & Bertsch, 2015). Biological control of snails has also been posited to circumvent the use of chemicals (Lin *et al.*, 2021). However, these have also been cautiously attempted due to several previous catastrophic releases of an organism in attempt to control another species (Marshall, 2019).

Other upcoming issues of concern in controlling the disease include the emergence of zoonotic reservoirs of the disease. *Schistosoma japonica* is well known to be zoonotic, infecting up to 40 species (Gordon *et al.*, 2019). However, other *Schistosoma* such as *Schistosoma intercalatum* and *S. mansoni* are primarily considered human infecting only.

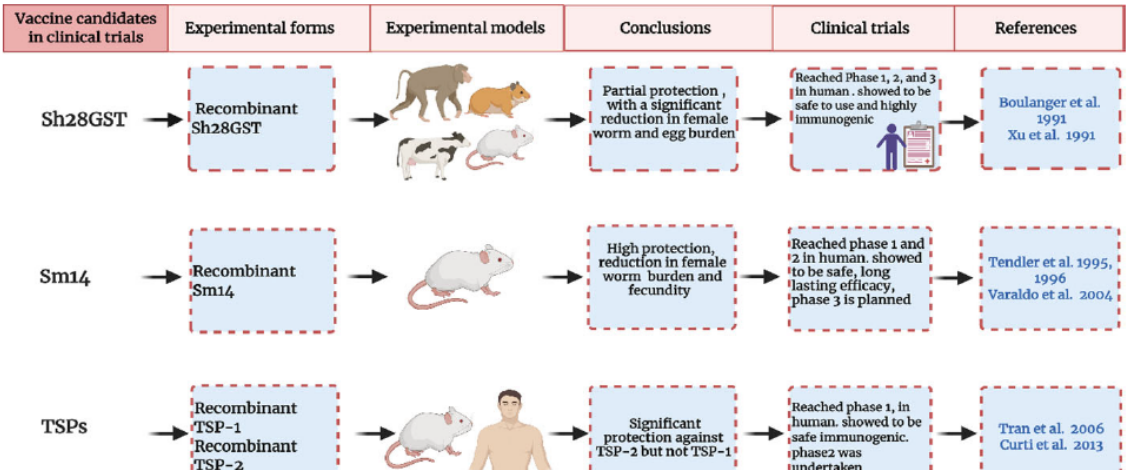


Figure 2.5: Showing the top vaccine candidates for schistosomiasis that are in clinical or pre-clinical trials. Image adapted from (Al-Naseri *et al.*, 2021).

2.2 Biology of host snails

The transmission of schistosomiasis depends on the distribution of their host snails. Snails belong to the phylum Mollusca of nearly 200,000 species, second only to arthropods in diversity (Strong *et al.*, 2008). They live across the planet and can be found on every continent except Antarctica, preferring aquatic habitats. The two host snails of schistosomiasis in Africa belong to the family Basommatophora, which are identified by their eye-position at the base of the tentacles rather than the tips.

2.2.1 *Biomphalaria*

Biomphalaria as a clade is discoid planorbids with a widespread distribution along the tropical Africa and America. There are thought to be 31 species in the genus, 12 of which occur in Africa the other 19 are found in South America (Brown, 2002). They are aquatic hermaphrodites capable of self-fertilisation, which allows a single individual to eventually colonise a new habitat. They lay eggs in batches of 5-40 in a jelly-like enclosure and can lay up to a thousand eggs in their lifetime. These eggs can hatch within 8 days and reach maturity within 4-7 weeks in optimal temperature and food availability conditions (WHO, 2010). They are known to live up to 18 months (Brown, 2002), during which they live on water plants or in areas with decaying organic matter, preferring waters with moderate pollution. They cannot resist dessication when exposed, as is the case in the dry seasons however some individuals may find refuge by burying themselves in mud and sealing their apeture with a layer of mucus (WHO, 2010).

Early experiments documented the ability of most species of *Biomphalaria* to transmit *S. mansoni* (DeWitt, 1954; Files & Cram, 1949). Of the African *Biomphalaria*, none are known to be completely resistant to infection with *Schistosoma*, unlike some of the New World species (Brown, 2002; Malek, 1985). However, the African clade - historically and presently, continue to be neglected in research, more than the American *Biomphalaria* species. Furthermore, there has been confusion as to the status of various putative species and sub-species of the African continent. An attempt to rectify this taxonomic enigma was made in the late 1950s by Georg Mandahl-Barth, who used a combination of morphological characteristics to re-classify African *Biomphalaria*. Mandahl-Barth proposed classifying the

African *Biomphalaria* into four groups: The *B. pfeifferi group, the B. choanphalla group,* the *B. sudanica group* and the *B. alexandrina* group. These groups also contained numerous species within each species-group (Mandahl-Barth, 1957a and b). However, more recently, the use of molecular tools has not been able to support this phylogeny. Particularly, DeJong *et al.* (2001) emphasised the close relation of three species, the *B. sudanica, B. choanphalla and B. alexandrina,* more so than even other species within their respective species groups, which led to the creation of a new clade, the 'Nilotic species complex'.

2.2.2 *Bulinus*

Freshwater snails of the genus Bulinus are widely distributed in Africa, especially the East African islands, the Middle East and some Mediterranean countries. They belong to the same Planorbidae family as *Biomphalaria* and thus the ecology of the genus is highly similar, despite the vastly different looking shell shape (Brown, 2002). These are, in contrast to the *Biomphalaria*, discoid shaped shell, higher ovate or sometimes turreted (Mandahl-Barth, 1957b). The genus is subdivided into four species groups each containing several species within them; these are the *B. forskalii* group*, B. africanus, B. truncates/tropicus* complex and the *B. Reticulatus* group (Fig. 2.6). In East Africa, *S. haematobium* are transmitted by Buliniids from the *B. africanus* species group (Brown, 2002). This includes four species in the region, namely, *B. globusus, B. africanus, B nasutus* and *B ugandae*. However, the last species is not known to transmit schistosomiasis in the area (Raahauge & Kristensen, 2000).

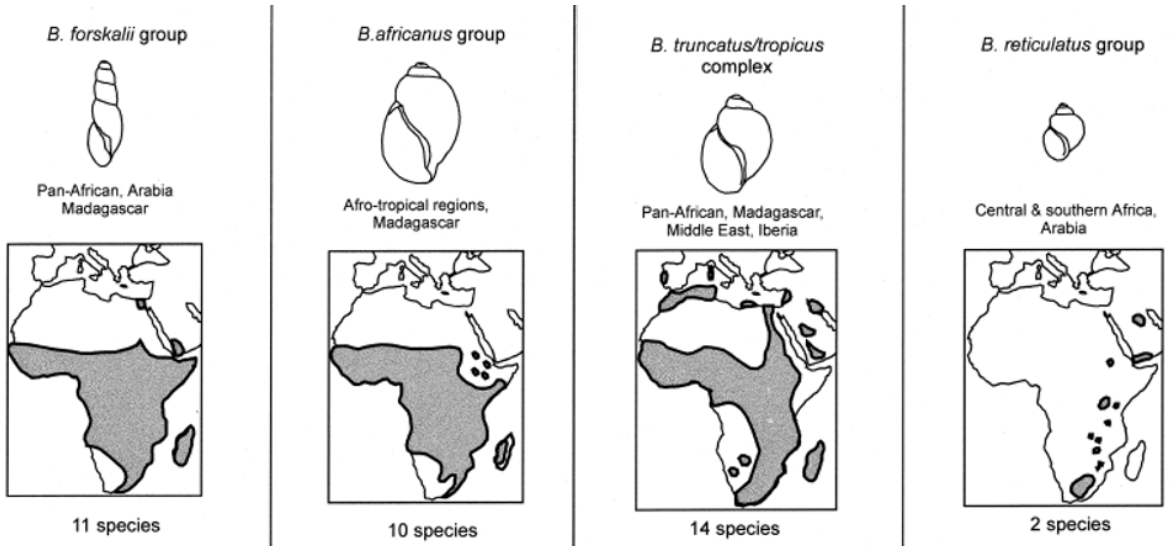


Figure 2.6: Distribution patterns of the four species of *Bulinus* in Africa. (Courtesy (Rollinson *et al.*, 2001)

Each species group contains several species within them. The hosts of human infecting *Schistosoma* fall under the *Bulinus africanus* complex of which three – *B. nasutus, B. globusus, and B. africanus* can be found in Kenya.

2.3 Snail ecology

Schistosomes utilise different snails as their host in different parts of the world. In the selected study area, the *Biomphalaria* and *Buliniids* are found in habitats such as rivers, dams or ponds, which they share with other taxa. The hosts are affected by several factors such as:

2.3.1 Temperature, light, water chemistry, conductivity, depth and type of water.

Temperature changes tend to be less drastic in water than in air, nevertheless they tend to affect the properties of the water (evaporation increases concentrations of salts within the water) and so this affects the distribution of the snails (De Kock, 1981). *Biomphalaria pfeifferi* has been found to have an optimal temperature range of about 20^{o}-29^{o}C (De Kock, 1981). Temperatures exceeding these limits have been shown to impair gonad development, which in turn reduces fecundity (Appleton, 1984). The influence of light has only been investigated by a handful of studies, mostly focused on *Biomphalaria glabrata* in Brazil. These studies found that mating increased in autumn and winter, and was depended on the schedule and intensity of illumination (Barbosa *et al.*, 1987; Pimentel-Souza *et al.*, 1988) while El-Emam (1982) had established that *B. alexandrina* could not be maintained in darkness.

The content of a body of water in terms of dissolved salts will generally affect the abundance of snail populations rather than determine their presence (Williams, 1970). A study in Lake Albert (formerly known as Lake Mwitanzige and Lake Mobutu Sese Seko), Uganda looking at, among other factors, total conductivity found high levels of total dissolved salts (44-700 IS) across their sampling sites. They found a negative correlation of densities of *B. sudanica* with increased water conductivity (Kazibwe *et al.*, 2006). *Biomphalaria pfeifferi* was shown to be especially susceptible to high magnesium concentrations even though they prefer medium to hard waters. Although some small subpopulations have slowly adapted to soft waters in certain areas (Brown, 2002). *Biomphalaria pfeifferi* was observed thriving in

sewage/wastewater purification ponds, where contamination by organic matter was high (between 60 and 1060mg/L) (Klutse, 1996). Concentrations can change through evaporation, as was the case in Niger, where increases in salinity due to higher temperatures caused *B. pfeifferi* to disappear (Gretillat, 1975). Decreases in salinity have also been reported as favouring the increases of *Biomphalaria,* as was the case after the construction of Diama Dam in Senegal (Vercruysse, 1994). *Biomphalaria alexandrina* was studied in Lake Maryut, Egypt, showing negative correlation with salinity, where the decrease in salt concentrations was linked to an increase in agricultural and waste discharge into the lake (Mohamed, 1978).

Biomphalaria have been shown to prefer shallow waters up to 7cm, or shorelines where the water depth does not exceed 40 cm and slow-flowing waters between 12-21cm/s. These values were ratified by studies on *B. sudanica* in Uganda (Kazibwe *et al.*, 2006) and *B. pfeifferi* in Tanzania (Utzinger & Tanner 2000; Utzinger, 1997). However, in South Africa, *B. pfeifferi* was found sheltering in the warm spring climate by residing in deeper waters (Appleton, 1979). A study in Tanzania also found *Biomphalaria pfeifferi* to prefer plant detritus and bedrock substratum (Utzinger & Tanner, 2000). They have also been reported to be more suited to permanent habitats, in isolated pools from perennial springs (De Kock, 1981). Brown (2002) concluded that in irrigation canals, periodical strong turbulent flow would more effectively wipe out snails, more than a steady rapid flow would. Water flow has been an important factor in the presence of snails, as reported in irrigation canals in Egypt, where it was found to be the key determinant in the presence of *B. alexandrina* (Yousif, 1993). Distribution of *B. pfeifferi* in Zimbabwe was also linked to water flow, alongside temperature (Woolhouse, 2009).

2.3.2 Aquatic plants

Plants in the environment assist the snails by shading them from intense sunlight and water currents while also providing a source of food and sheltered site to deposit their eggs (Brown, 2002). International trading of aquatic plants has also been accredited with assisting in the spread of planorbid snails (Pointier *et al.*, 2005). *Biomphalaria* species from different parts of the world have been associated with different vegetation (Kader, 2001; Yousif, 1998). A study in Nigeria showed that the presence of *Biomphalaria pfeifferi* correlated with the presence of aquatic macrophytes of the family Graminiae (mainly *Paspalum* spp), as well as *Acroceras zizanoided*, *Alternanthera sessilis, Commelina* and *Ludwigia leptocarpa* (Ndifon, 1989). Vegetation may also impact the transmission of schistosomiasis, as reported in Bahia, Brazil where it was found that higher prevalence occurs in areas with latossolo soil and

transitional vegetation (Bavia *et al.*, 1999). However, too much shade from hanging vegetation could factor in a lack of snails, probably through indirectly removing their diatomatious food (algae). It was found that *B. Pfeifferi* preferred minimal shading of about 0-25% (Ndifon, 1989). Shade may be unfavourable as it has been suggested to be a form of biological control (Loreau & Baluku, 1991).

2.3.3 Predators and competitors

Gastropods are integral in the food chains in which they are preyed on by insects, annelids, crustaceans, birds, humans as a delicacy, and even other gastropods (Brown, 2002). Their simple body layout, combined with their ability to self-fertilise give them the ability to quickly multiply and colonize new environments; yet it makes them easy targets for larger, and/or more evolutionary "equipped" taxa (Vermeij, 1978). In the study area, of the Kenyan Lake Victoria region, many macroinvertebrates co-exist with the *Biomphalaria* and Bulinid snail hosts, of which, five taxa have been studied in Africa as predators: leeches (glossiphonidae), sciomyzoid flies (diptera), waterbugs (hemiptera), crabs and the introduced crayfish *Procambarus clarkia (*decapoda*)*. Leeches are predaceous and feed on several macro-invertebrates, including annelids (Oligochaeta), non-biting midge (Chironomid) larvae and snails (Mollusca) (Sawyer, 1986). However, there is substantial record of the family Glossiphonidae feeding exclusively on snails (termed *malacophagous*), including the genera *Glossiphonia and Helobdella* (Crewe, 1973; Davies *et al.*, 1997; Harry & Aldrich, 1958; Michelson, 1957). *Glossiphonia* are so adapted such that they may even be able to follow the mucus trail of the snails to locate them (Sawyer, 1986). However, the genus is not found in Africa, while *Helobdella,* another genus of leech is well represented on the continent (Day & de Moor, 2002). *Helobdella conifera* is found across Africa, feeding readily on Pulmonates, though not exclusively (Appleton *et al.,* 2004). Laboratory experiments showed that the leech could consume up to 60 snails in their lifespan of 280 days (0.21 snails/day) when fed *Helisoma duryi* (Planorbidae). They would not attack for the first 11 days and preferentially target juvenile snails of 4-10mm (Davies *et al.*, 1997). They have also been found to be more adept at hunting indigenous *Bulinus tropicus* (Planorbidae) and *Lymnea natalensis* (Lymnaeidae) than exotic *Physa acuta* or *Aplexa marmorata* (Physidae) due to the Physidae's ability to evade attacks (Wilken & Appleton, 1991).

Marsh flies (Sciomyzidae) are a family whose larvae are known to feed on snails, so much so they are often called snail-feeders. Some species survive on an exclusively malacophagous diet. They were suggested as biocontrol agents to tackle schistosomiasis (Berg, 1953). Their

biology was elucidated in a rapid interest in the ‘80s and ‘90s where their feeding efficacy was studied in Africa (Barraclough 1983, 1985; Maharaj, 1992). These, studies revealed prey choice of up to ten snail species; and method of attack which is to sink their mouth-hooks to the soft parts of the snail, killing the snail within minutes (Berg, 1953). However, the need for low over-hanging vegetation for the flies to deposit their eggs would be lacking in areas of transmission, either due to human or animal activity, and so the method of control was deemed “probably ineffective” and has not been explored since (Appleton *et al.*, 2004).

Belostomatidae are another well-known snail-feeding family. Studies on the giant water bug’s snail feeding habits were documented in Egypt using the pan-African *Limnogeton fieberi*, which feed on pulmonate and prosobranch through all its five instars. Experimentally, *L. fieberi* has been shown to progressively kill more snails, from a mean of 3.2 at 1st instar up to 30 snails per larvae at the 5th instar. In terms of daily kills, the bugs killed a mean of 2.3 *B. alexandrina* per day (Voelker, 1968). Another water bug known for malacophagy is the Afro-Asian *Appasus grassei* (Polhemus, 1995), which was experimentally shown to non-specifically feed on *Bulinus tropicus, Physa acuta* and *Aplexa marmorata* (Baijnath, 1999).

2.3.4 Other environmental factors

Environmental factors that influence the aquatic habitats, the host-snails residing within them, their predators, and the parasite are not well known. Furthermore, the link between the use of agrochemicals and snail populations has not been established. Studies have estimated that for the current population trend, fertiliser use is expected to increase two- to five-fold by 2050 (Tilman *et al.*, 2001). Studies have also sought to assess the risks of these chemicals which are often found in tandem with others (Altenburger, 2013; Relyea 2009); and the consequence of chemical mixtures on individual species has been investigated (Anderson, 2002; Boone & James 2003; Fairchild, 1994; Hayes *et al.*, 2006; Relyea, 2009).

Yet understanding the overall effect on the aquatic communities and the ecosystem is much more challenging (Altenburger, 2013; Relyea 2009). In the ecosystem within this study areas, the *Schistosoma*-host snails interact with various other species. Studies have shown that fertilizers and herbicides can both directly and indirectly, respectively influence the density of the snails, by increasing algae which is a food source for snails (Halstead, 2014; Rohr *et al.*, 2008). Furthermore, densities of *Schistosoma*-host snails have also been recorded to increase when ecological factors disadvantage their predators, such as the case when Diama Dam was constructed in Senegal, restricting the movement of the native

Macrobrachium (Savaya Alkalay *et al.*, 2014). Kenya saw an increase in the amount of pesticides imported from 6400 tons in 2015 to 15,600 in 2018 (Biodiversity and Biosafety Association of Kenya (BIBA-K), Kenya Organic Agriculture Network (KOAN), Resources Oriented Development Initiatives (RODI Kenya) and Route to Food Initiative (RTFI), 2019). Therefore it is important to investigate the implications of agrochemical pollution on the ecosystem of the snail, which may allow for either an increase or decrease of population of the snails directly as this would have implications in disease transmission and control.

2.3.5 SPEARpesticides Bioindicator

Bioindicators are a surveillance tool in which monitoring of aquatic systems is done through observation on the organisms in the environment of interest. An idea first conceived over a century ago that related the occurrence of certain taxa to contamination with organic matter (Kolkwitz & Marsson, 1902). Since then, several other bioindicators were developed that mostly relied on taxonomic properties of the freshwater macroinvertebrate community or on specific species as indicators (Cairns & Pratt, 1993). However, these were found to not be stressor specific, nor was their application transferrable from place to place due to natural changes in the taxa present (Bonada *et al.,* 2006; Menezes *et al.,* 2010). SPEARpesticide is the acronym for a bioindicator that was developed to use data available on the physiological and biological traits of local species to create a binary system where these species are either "at risk" for pollution or "not at risk". This allows it to overcome the challenges of other bioindicators as it can be locally adapted and could be tuned to be stressor specific (Von Der Ohe & Liess, 2004). The bioindicator expresses the proportion of taxa classified as vulnerable to pesticides ("SPEcies At Risk") to the overall number of macroinvertebrates found at a site, where a low proportion of vulnerable taxa indicates an impact from pesticides. The classification of macroinvertebrates in vulnerable species at risk ("SpeAR") and species not at risk ("SpeNotAR") is based on four ecological traits concerning (1) the physiological sensitivity to pesticides (s-value), (2) the ability for population recovery through reproduction (generation time), (3) the ability for recolonization (migration), and (4) the probability of exposure (presence in the stream during pesticide spraying season. Therefore, a comprehensive trait data base has been established for temperate macroinvertebrate communities (Indicate 1.0.0, http://www.systemecology.eu/indicate). Taxa considered sensitive in all four ecological traits are classified as vulnerable (Liess and von der Ohe 2005). The fraction of log-transformed abundance of sensitive species is calculated by:

$$\%\mathrm{SPEAR}_{\mathrm{abundance}} = \frac{\sum_{i}^{n} \log(x_i + 1) y_i}{\sum \log(x_i + 1)}$$

where n is the total number of taxa in the sample, x_i is the abundance of a taxon i, and y_i is 1 if taxon i is sensitive, or 0 if not (Schäfer *et al.*,2013). The bioindicator was developed in Germany, using data from macroinvertebrates collected from German streams (Von Der Ohe & Liess, 2004). To determine the s-value (the physiological sensitivity to pesticides) for aquatic invertebrates. acute toxicity experiments were conducted which allowed the relative sensitivities of each species as compared to that of *Daphnia magna* to be calculated (Fig 2.7). In doing so, it was discovered that Planorbidae snails were especially tolerant to pesticide pollutants. This taxa holds the genera that are host snails of *Schistosoma*. Therefore, it led to the hypothesis that these host snails could benefit in polluted environments due to a reduction in sympatric macroinvertebrates that would be either competitors or predators of the snails.

Order/Suborder	Family	Genus	Species	$S_{organic}$[a]	(su|re|te)	S_{metal}[a]	(su|re|te)
Annelida							
Hirudinea				−0.60[b]	(18|18|18)		
	Erpobdellidae			−0.41	(13|13|13)		
Oligochaeta				−1.10[c]	(20|22|40)	−0.80[c]	(35|37|92)
	Lumbriculidae			−1.40[b]	(9|10|11)	−0.51	(12|17|27)
	Tubificidae			−0.93[b]	(6|7|15)	−0.80[c]	(20|22|48)
Crustacea							
Amphipoda				0.16	(95|104|200)	−0.30	(18|19|29)
			Gammarus fasciatus	0.19	(30|34|68)		
			Gammarus lacustris	0.32	(22|23|51)		
			Gammarus pseudolimnaeus	0.14	(11|13|25)		
			Gammarus pulex	0.04	(22|24|45)	−0.03	(5|5|9)
Cladocera[d]				0.17[c]	(143|179|420)	0.48[c]	(70|91|280)
	Daphnidae[d]			0.20[c]	(136|172|413)	0.49[c]	(69|89|275)
		Ceriodaphnia		0.28[b]	(49|68|163)	0.62[c]	(17|25|77)
			Ceriodaphnia dubia	0.39[c]	(41|59|152)	0.64[c]	(10|16|62)
		Daphnia[d]		0.20[b]	(64|78|181)	0.50[c]	(34|43|145)
			Daphnia culcullata	0.08	(12|12|22)		
			Daphnia pulex	0.20[c]	(42|55|146)	0.38[c]	(18|27|112)
		Moina		−0.22	(11|12|32)	0.40	(9|12|39)
			Moina macropoda	−0.17	(10|11|31)	0.18	(5|8|13)
		Simocephalus		0.24	(12|14|37)	0.32[b]	(9|9|14)
			Simocephalus serrulatus	0.25	(8|8|8)		
Decapoda				−0.08	(29|36|103)		
	Astacidae			−0.57	(13|13|40)		
			Oronectes nais	−0.41	(10|10|34)		
	Palaemonidae			0.26	(15|22|57)		
Isopoda				−0.56[b]	(40|49|57)	−1.22[b]	(7|10|24)
			Asellus aquaticus	−0.17	(40|49|57)	−1.55[b]	(5|8|14)
			Asellus brevicaudus	−0.56	(12|17|17)		
Mollusca							
Basommatophora				−1.23[c]	(89|127|171)	−0.82[c]	(23|28|69)
	Lymnaeidae			−0.64	(42|71|109)	−0.93[c]	(12|17|27)
			Lymnea acuminata	−0.93	(11|15|26)		
			Lymnea stagnalis	−0.62	(22|47|66)		
	Physidae			−1.64[c]	(36|45|46)	−0.35	(6|6|34)
			Physa acuta	−1.88[c]	(25|34|34)		
	Planorbidae			−1.94[c]	(11|12|18)		
Eulamellibranchia				−2.09[c]	(21|24|41)	−0.33	(7|8|13)
Prosobranchia				−1.82[b]	(8|9|21)		
	Viviparidae			−1.50[b]	(7|8|19)		
Insecta							
Anisoptera[f]				−0.96	(12|12|16)		
	Libellulidae			−1.53[c]	(8|8|12)		
		Orthetrum		−1.75[c]	(7|7|11)		
Coleoptera				−1.15[c]	(25|26|36)		
	Dytiscidae			−0.81[c]	(10|11|15)		
	Haliplidae			−1.83	(7|7|12)		
		Peltodytes		−1.95	(6|6|11)		
	Hydrophilidae			−0.89[c]	(7|7|8)		
Diptera				−0.35[c]	(429|573|958)	−1.57[c]	(35|41|81)
	Chironomidae			−0.39[c]	(159|213|316)	−1.53[c]	(28|32|69)
		Chironomus		−0.33[c]	(110|141|202)	−1.50[c]	(26|30|66)
			Chironomus tentans	−0.19	(28|34|46)	−1.36[b]	(6|8|19)
			Chironomus thummi	−0.30	(36|46|63)	−1.86[a]	(7|7|21)
		Tanytarsus		−0.36	(28|43|56)		
			Tanytarsus dissimilis	−0.34	(23|35|48)		
	Culicidae			−0.30[c]	(259|349|631)	−1.73[a]	(7|9|12)
		Aedes		−0.29[b]	(114|137|224)		
			Aedes aegypti	−0.14	(28|45|87)		
			Aedes cantans	−0.18	(13|17|21)		
			Aedes punctor	−0.34	(13|13|13)		
			Aedes vexans	−0.07	(13|15|15)		
Ephemeroptera				−0.30	(38|42|53)	−1.61[b]	(5|8|14)
	Baetidae			−0.25	(33|37|46)		
		Baetis		0.02	(7|9|11)		
		Cloeon		−0.32	(26|28|35)		
Heteroptera				−0.56[c]	(33|35|46)	−1.63	(5|6|10)
	Corixidae			−0.29	(18|20|28)		
		Corixa		−0.31	(13|13|13)		
		Sigara		−0.24	(5|7|15)		

Figure 2.7 An excerpt of the relative sensitivities of various taxa to pesticides, organic or metallic pollutants in relation to that of *Daphnia magna*. The sensitivity of molluscs can be noted to be exceptionally high, with planorbids being the second most tolerant taxa to pesticide pollution (Von Der Ohe & Liess, 2004).

CHAPTER THREE

HOW PESTICIDES AFFECT *SCHISTOSOMA*-HOST SNAILS

Abstract

Schistosomiasis is a severe neglected tropical disease caused by trematodes and transmitted by freshwater snails. Snails are known to be highly tolerant to agricultural pesticides. However, little attention has been paid to the ecological consequences of pesticide pollution in areas endemic for schistosomiasis, where people live in close contact with non-sanitized freshwaters. In complementary laboratory and field studies on Kenyan inland areas along Lake Victoria, I show that pesticide pollution is a major driver in increasing the occurrence of host snails and thus the risk of schistosomiasis transmission. In the laboratory, snails showed higher insecticide tolerance to commonly found pesticides than associated invertebrates, in particular to the neonicotinoid Imidacloprid and the organophosphate Diazinon. In the field, I demonstrated at 48 sites that snails were present exclusively in habitats characterized by pesticide pollution and eutrophication. Our analysis revealed that insensitive snails dominated over their less tolerant competitors. The study shows for the first time that in the field, pesticide concentrations considered "safe" in environmental risk assessment have indirect effects on human health. Thus I conclude there is a need for rethinking the environmental risk of low pesticide concentrations and of integrating agricultural mitigation measures in the control of schistosomiasis.

3.1 Introduction

Schistosomiasis, also known as, bilharzia, is among the tropical diseases with the highest impact on socio-economic development, only exceeded by malaria. Approximately 218 million people are infected worldwide (WHO, 2018). Infection has been strongly associated with long-term disabilities (King, 2008a). The number of deaths due to schistosomiasis is poorly documented with estimates ranging between 11,700 (Lozano, 2012) to 280,000 each year (King, 2008b) because of hidden pathologies such as liver and kidney failure (Colley, 2014). Schistosomiasis is caused by flatworms of the genus *Schistosoma* which parasitize humans as their definitive host (supporting the adult life stage of the parasite). The intermediate hosts are freshwater snails of the family planorbidae which release infective larval stages (cercariae) into the water. Transmission occurs when humans are exposed to water containing infected host snails; direct infection from person to person is not possible

(CDC, 2018). People are infected during routine agricultural, domestic, occupational and recreational activities, which expose them to infested water. Over 80% of afflicted people live in sub-Saharan Africa (Hotez, 2014), but the disease concerns public health in most (sub)tropical countries worldwide (Hotez, 2014) and has recently established in Europe (Pennisi, 2018). Control strategies against schistosomiasis focus on the treatment with praziquantel that kills the adult worms in the human host. However, even mass drug administration does not prevent re-infection in infested water, and schistosomiasis has been observed to rebound within short time (Gurarie, 2018). For the sustainable control of schistosomiasis, it is also essential to interrupt the infection cycle by control of the intermediate hosts (Chadeka, 2019; Lo, 2018; Secor, 2014). Host snails are susceptible to predation by organisms such as shrimps (Sokolow, 2014) and ostracods (Yousif, 2013) which have been applied as biological control agents. Additionally, host snails are susceptible to competition from other snails and insects that feed on periphyton (microbes attached to surfaces), detritus and water plants (Barbosa, 2014; Mone, 2003; Yeung, 2012). Spreading of schistosomiasis has been often linked to the loss of biodiversity and the ecological degradation of freshwater habitats (Johnson, 2010; Secor, 2014; Sokolow, 2017). The findings suggest that host snails are significantly controlled by antagonistic species in natural habitats but that this ecosystem service is sensitive to anthropogenic impact. Therefore, it is essential to identify key environmental factors that drive the interactions of host snails with their associated community.

Recently, agricultural pesticides have returned to the focus of public attention as causes for the worldwide decline in insects and biodiversity (Beketov, 2013, Geiger, 2010; Liess, 2005). Tropical regions, characterized by extensive agriculture and heavy rainfalls, are known areas of endemicity of schistosomiasis. In such conditions there is a high risk of surface run-off that washes pesticides from agricultural fields into adjacent freshwater systems (Liess, 1999). However, information on pesticide concentrations in tropical freshwaters and their effects on the macroinvertebrate community are often fragmented and inadequate (London, 2005; Musa, 2011). In temperate latitudes, snails are known as one of the macroinvertebrate taxa being most tolerant to pesticides (Von Der Ohe, 2004). In mesocosms, high concentrations of insecticides and herbicides favoured host snails indirectly through the reduction of predators and through the replacement of suspended algae with periphyton that serves as food for snails (Halstead, 2018). Additionally, even low levels of agricultural pesticides result in a typical replacement of sensitive macroinvertebrates by more tolerant taxa in mesocosms (Van den

Brink *et al.*, 2000) and in natural streams (Knillmann, 2018, Liess, 2005). These effects are usually driven by insecticides that are most toxic to many macroinvertebrates (Liess, 2005). Therefore, I hypothesized that pesticide pollution may favour highly tolerant snails that host human-pathogenic schistosomes over their more sensitive natural enemies and thus increase the risk of schistosomiasis transmission.

3.2 Materials and methods

3.2.1 Acute toxicity tests

I investigated acute insecticide sensitivity for all macroinvertebrate taxa that could be found in sufficient quantities in October / November 2018 from 6 sites in the study area. Site selection was based on host snail availability, high macroinvertebrate diversity, and low presumed pesticide pollution as indicated by buffer strips to minimize testing populations that may have developed pesticide resistance (Becker & Liess, 2017). Test organisms were collected using sweep nets, standard pint dippers and snail catchers; they were sorted and identified to family level in the field. The organisms were placed in plastic lunch boxes filled with water from the sampling site and aerated using battery-operated air pumps. The containers were cooled in a portable fridge at 18°C in order to prevent mortality during transport to the laboratory. The organisms were acclimatized to test conditions overnight. Tests were performed in a shaded screen-house with temperatures ranging from 20 to 33°C. Commonly applied agricultural insecticides comprise three major classes with distinct modes of action: Organophosphates / carbamates, neonicotinoids and pyrethroids. If sufficient test organisms were available, I tested the acute sensitivity to one of the most toxic substances among the organophosphates (diazinon) and neonicotinoids (imidacloprid) measured at the study sites, respectively. To increase environmental realism, I applied local plant protection products containing the active substance and additional carriers that might have affected the toxicity.

Tests were performed according to the Rapid Test protocol for field-collected organisms (Blaise & Férard, 2013) with minor modifications. Imidacloprid was applied as a 70% wettable granule formulation (Loyalty, manufactured by Shandong United Pesticide Industry Co., Ltd. Jinan city, China) and diazinon was applied as a 60% emulsified liquid formulation (Diazol, repacked and distributed by Laibuta Chemicals Ltd). Fresh stock solutions were prepared a few hours before each test dissolving the formulations in a 1:1 mixture of bottled water and activated carbon filtered stream water. This mixture was a compromise to minimize

adverse effects from water to which the organisms had not been adapted, and to minimize potential effects from residual toxicity and from dissolved solids in the stream water which can absorb pesticides. Stock solutions of 165 mg active substance/L were left to stir overnight in amber vials; no additional solvents were applied.

Test organisms were exposed to 6 test pesticide concentrations including a control. For each taxon test concentrations were selected from the following geometric series such that they covered the expected range of a partial response from < 5 % mortality in the lowest concentrations to > 95 % mortality in the highest concentration: 0.001; 0.004; 0.014; 0.055; 0.209; 0.792; 3.01; 11.4; 43.5, 165 mg/L.The ranges expected to result in a partial response were identified from data bases (Lewis *et al.*, 2019; USEPA, 2019) and previous studies (Becker & Liess, 2017) for related taxa and substances. The tests were performed in 100ml glass vessels containing up to 5 individuals of the same species (predators were kept individually to avoid cannibalism). The test medium was constantly aerated using aquarium pumps connected to glass pipettes via a silicone tube. Only the glass pipette had contact with the test solution and the air flow was controlled through a clamp on the silicone tube. After 24 h and = 48 h, mobile, immobilized and dead individuals were counted. Individuals were considered immobilized when no movement was observed within 10 s of undisturbed observation or after probing with a rod; fanning of gills was not considered movement.

3.2.2 Field sampling

48 study sites located in Homa Bay, Kericho, Kisii, Kisumu, Migori and Nyamira in Western Kenya were investigated from September – November 2017. The aquatic habitats were chosen from areas characterised by different types of land use and crops grown, identified using aerial photos from Google Maps. I classified the sampling sites according to habitat types (major tributary, minor tributary, irrigation channel, oxbow lake, reservoir or rice field) and the surrounding dominant land use within 50-100 m (natural, agricultural, semi-urban, urban or industrial). Agricultural land use was classified by farm type, subsistence or irrigation schemes and crop type (maize, tea, sugarcane or rice).

Streams, rivers and oxbow lakes were sampled across a 50-metre section whereas dams and irrigation schemes were sampled at four sub-sites. The aquatic habitats were surveyed for the presence of submerged, emerging or floating vegetation as well as algal bloom and the percentage of detritus cover. Depth was measured at the bank at point of sampling using a metre rule or was indicated as >1m. Flow velocity was estimated with the drift approach. Additionally, I measured physicochemical parameters (temperature, conductivity, pH,

dissolved oxygen, carbonate hardness, ammonium, phosphate, nitrate, nitrite and nitrite) and the turbidity on site using cholorimetric test kits (MACHEREY-NAGEL Quantofix, Düren, Germany), a turbidimeter (WTW TURB 355 IR, Weilheim, Germany), a multi-measurement probe (EXTECH ExStick EC500, Boston, USA) and an oxygen probe (EXTECH ExStick DO600, Boston, USA).

For pesticide analyses, grab samples were taken using pre-cleaned glass beakers. Briefly, oven dried 500 mL beakers were rinsed three times with the sample water and filled up to the top. After suspended solids settled, 1 mL aliquots were taken into five 2 mL autosampler amber glass vials (Phenomenex, Germany) using a volumetric pipette. All samples were immediately stored in a portable freezer (Waeco Compressor Cooler Box - 50 litres #CF-50) at -4°C and transferred to the laboratory where they were stored at -20°C until analysis. For quality control, sampling and trip blanks were taken during each sampling campaign.

Macroinvertebrates were sampled along four equal sections of the water body. Banks were sampled in a criss-cross fashion along the sampling points using littoral sweep nets, dippers and snail catchers; a standardised sampling procedure was predetermined to collect macroinvertebrates comprehensively within the different microhabitats and habitat types. In brief, each site was sampled for 30 minutes by two persons in parallel. Collected macroinvertebrates were sorted and counted in white plastic trays and preserved in 70% ethanol. Some host snails were transported to the laboratory and checked for *Schistosoma* infection. The snails were kept individually in a 24-well plate (Nunc 142475 Nunclon) and exposed to sunlight for a minimum of 30 minutes, and shed cercariae were = identified under a dissecting microscope (Zeiss AxioCam5 100-400x) and an identification key for cercariae (Frandsen and Christensen, 1984). Macroinvertebrates were identified under a dissecting microscope (Zeiss AxioCam5 100-400x) to the lowest taxonomic level possible with the available identification keys (Brown, 2002; Day *et al.*, 1999; Day, 2001a, 2001b; Day, 2002; Day & de Moor 2002a, 2002b; de Moor, 2003a, 2003b; Harrison, 2009). Based on these data I calculated the following biological indices: the overall macroinvertebrate individual number, the species richness, Pielou's species evenness, the Shannon index for species diversity, the ASPT indicator for stream health from the South African Scoring System SASS (Dickens & Graham, 2002), and the dominance (relative abundance) of potential predator and competitor species of the host snails. I considered all taxa as potential predators that comprise more than a marginal proportion of predatory species in the study region that may feed on freshwater snails or their eggs. Similarly, I considered all taxa as potential competitors that

comprise more than a marginal proportion of periphyton feeders or herbivores in the study area (Tab. S6).

3.2.3 Ethical considerations

This study was conducted according to best research practice and ethics (Kelley, 2013). The sampling sites will be preserved as much as possible during the process and prevent contamination between sites. Sterilisation was done by spraying either 70% Ethanol or 50% Acetone on any equipment used. Macro-invertebrates not collected as specimen were returned to the habitat. The local communities were engaged to explain what work I do, and permission requested to enter sites that belong to somebody, or are used for agriculture, water collection and other domestic purposes.

Many of the rivers of the areas are used for washing and cleaning and so areas were approached with caution, in secluded spots and alert others to my presence. The rights of people to access their communal water supplies, as well their properties must be respected (Kelley *et al.*, 2013).

3.2.4 Analysis of Pesticides in water

Details on the analysis of pesticide residues in water samples have been described by Kandie *et al.* (2020a). In brief, 25 µL of an internal standard solution containing 40 isotope-labelled compounds (40 ng/mL9, 25 µL of methanol and 10 µL of 2MNH4-formate buffer (pH 3.5) was added to each sample prior to instrumental analysis. Analysis was performed using high-performance liquid chromatography (HPLC, Ultimate 3000 LC) coupled to high resolution mass spectrometry (HRMS, QExactive Plus MS) from Thermo Scientific. The sample (100 µL) was directly injected for chromatographic separation (Phenomenex Kinetex c18 EVO, 50 x 2.1 mm, 2.6 µm particle size), equipped with a pre-column (5.0 x 2.1 mm) and 0.2 µm in-line filter using a methanol / water gradient containing 0.1% formic acid. Heated electrospray ionisation (ESI) was performed for both the negative and positive modes with combined full scan run (100−1500 m/z) at a nominal resolving power of 70,000 (referenced to m/z 200) and data-independent MS/MS fragmentation (DIA) at a nominal resolving power of 35,000. An isolation mass window of m/z = 50 (m/z range 122 - 860) or m/z = 260 (m/z range 860 – 1370) was used in DIA analysis. Matrix matched calibration standards were prepared for 11 calibration levels (ranging from 1 to 2,000 ng/L) using 1 mL filtered water from a pristine reference stream (Wormsgraben, Harz Mountains, Germany). Quantification of detected pesticides was performed using isotope –labeled internal standards of compounds with closest retention time to the target compound. Data evaluation was

performed using MZmine (Version 2.38 http://mzmine.github.io/) and trace finder (Thermo, version 4.1 https://www.thermofisher.com/ke/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-software/lc-ms-data-acquisition-software/tracefinder-software.html).

3.2.5 Data analysis

All data were analyzed using the software R 3.5.2 (R Core Team, 2018). From the mortality observed in the acute toxicity tests I calculated the acute lethal median concentrations after exposure for 24 h ($LC50_{24h}$) with 4-parameter log-logistic non-linear regression available with the package drc 3.0-1 (Ritz, 2015). The parameters for the upper and lower boundary were fixed to 1 and 0, respectively. If a taxon had been tested at more than one date or from more than one site, data were merged prior to the analysis. Tests which showed > 30 % control mortality were excluded from analyses. The resulting LC_{50} values were ranked in ascending order to obtain the species sensitivity distribution (SSD). This increase in the proportion of affected taxa with pesticide concentration was described using a quasibinomial generalized linear model (GLM) with a logit link function.

For all GLMs in this publication, *p*-values were obtained from likelihood-ratio tests that compared each model to a null model without the environmental variable. Depicted means and 95 % confidence intervals were extracted from (generalized) linear models using the package effects 4.1-0 (Fox & Weisberg, 2018). Normally distributed residuals and homoscedasticity were confirmed using normal Q-Q plots and plotting residuals vs. fitted values; GLMs were inspected using scaled residuals available with the package DHARMa 0.2.0 (DHARMa, 2018). The effects of each environmental variable measured on the incidence of host snails were analyzed using one-way binomial GLMs (binary regression) with a complementary log-log link function that allows for a non-symmetric dose-response curve. The effects of each environmental variable measured on the density of existing host snail populations were analyzed using one-way GLMs with a zero-truncated negative binomial distribution of residuals and a log link function available with the package VGAM 1.0-6 (VGAM, 2018). This way the overdispersion and with the missing possibility for the population density to be zero was dealt with. Since many effects on the population density of host snails were driven by a single site, (site 39) with extraordinarily high numbers of host snails and other macroinvertebrates, the analysis on the effects of host snail density with that site excluded was repeated. Only those environmental variables with $p < 0.05$ were

considered in further analyses. However, as explained above, the population density after site 39 had been excluded.

Environmental variables that explained the incidence or population density of host snails were combined in a hurdle model available with the package pscl 1.5.2 (Zeileis, 2008). Hurdle models consist of two connected generalized linear models to simultaneously fit the incidence (zero part) and the population density (count part). Prior to modeling, the environmental variables were standardized (normalized and centered) to make the model parameters comparable. Environmental variables with $p < 0.05$ explained the incidence were incorporated in the zero part of the model (binomial GLM with complementary loglog link), and environmental variables that explained the population density were incorporated in the count part (zero-truncated negative binomial GLM with log link). To avoid over-fitting, an additive model without interactions was applied. Then all non-significant environmental variables based on a likelihood ratio test (backward elimination) was sequentially removed. Each time a variable had been removed, I started testing again with the least-significant of the remaining variables according to the model statistics.

Additionally, I fitted a hurdle model with all the environmental variables that on their own significantly explained the incidence or the population density included in both the zero and the count part. Hurdle models consist of two connected generalized linear models to simultaneously fit the incidence (zero part) and the population density (count part) (Zeileis, 2008). I removed all non-significant effects from this full model in a stepwise backward-elimination process and then sorted the remaining effects based on the magnitude of their regression coefficients. Due to multicollinearity, selecting a single minimum adequate model can lead to different results depending on the method of model selection (Mac Nally, 1996). Therefore, this model was subjected to hierarchical partitioning. Because this procedure is currently not available for hurdle models in R, I extended the code of the function hier.part from the package hier.part 1.0-4 (hier.part, 2013). The modified function started with a null model and each step included an environmental variable to both the zero and the count part at the same time. The improvement of the goodness-of-fit that resulted from the inclusion of an environmental variable was quantified using the log-likelihood.

Relations among the environmental explanatory variables were visualized using a principal component analysis (PCA) available with the function prcomp in basic R. A PCA reduces complexity by combining correlated environmental variables to few “supervariables” called principal components. The data were standardized prior to the analysis. Additionally, the

association of the second principal component with the log-transformed number of macroinvertebrate individuals was analyzed using ordinary one-way linear regression.

The effect of pesticide pollution (TU_{max}) on the overall community composition consisting of grazers, potential predators and other macroinvertebrates was analyzed using a permutational multivariate analysis of variance (PERMANOVA) available with the package vegan 2.5-4 (vegan, 2019). I also investigated the effect of pesticide pollution on the taxonomic composition of potential predators using a PERMANOVA. To investigate effects of the species composition of potential predators on the grazer composition, I performed a PCA on the predator composition. The first to fifth principal component was then fitted vs. the dominance of snails within grazers using quasi-binomial GLMs with a logit link function to account for the possibility of overdispersion. The proportions were weighted with the numbers of observed grazers. Similarly, the effects of pesticide pollution on the dominance of all grazers, of potential predators, and of snails within the guild of grazers were analyzed using quasi-binomial GLMs with a logit link function. The proportions were weighted with the numbers of observed individuals or of observed grazers, respectively. All data were analyzed using the software R 3.5.2 (R Core Team, 2018).

3.3 Results

I studied how pesticide pollution and additional environmental factors affect the macroinvertebrate community composition in a typical endemic region of schistosomiasis. For this, I sampled 48 freshwater sites in the Kenyan Lake Victoria Basin (Fig. 3.1). The habitats ranged from small and medium-sized streams to irrigation channels, oxbow lakes, reservoirs and rice fields, and thus covered the main inland transmission sites in the study area (Sang, 2014). Each site was monitored once during the rainy season in October 2017. To confirm the hypothesized high pesticide tolerance of host snails, I collected host snails and other common invertebrates and tested their acute sensitivity to two insecticides covering different modes of action.

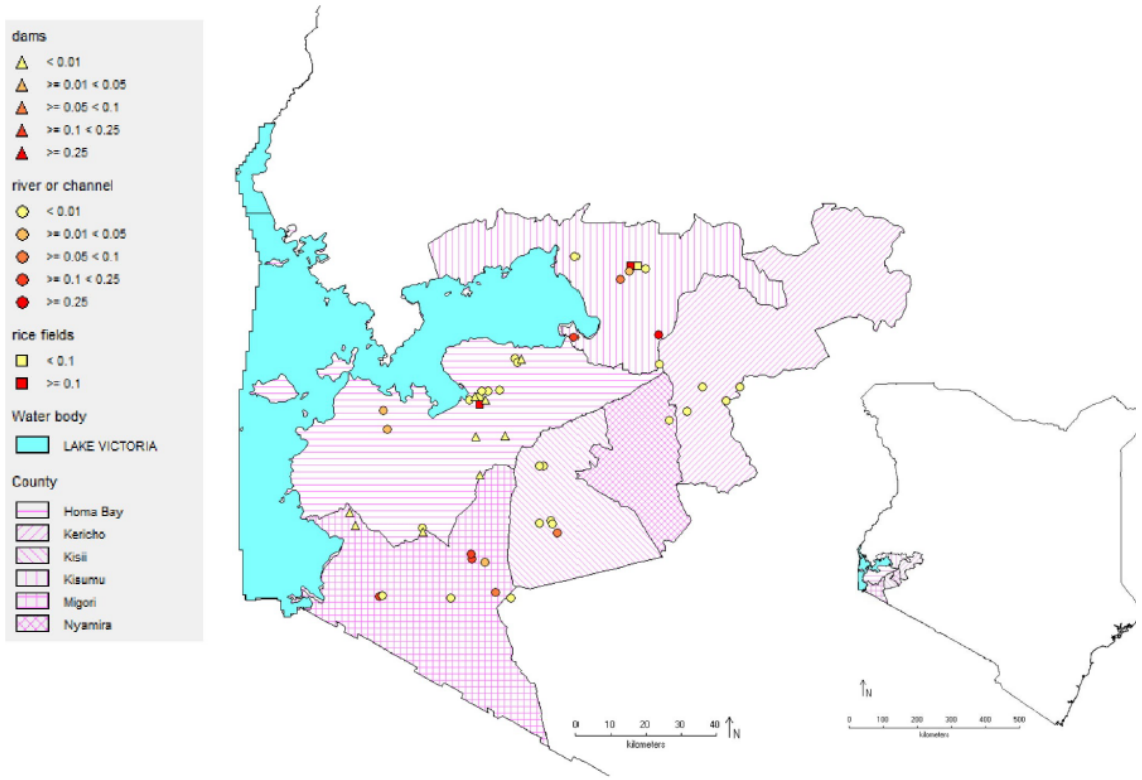


Figure 3.1: Dominance of host snails of the pathogens of human schistosomiasis among the study sites. Sites were depicted by the shapes of the icons as either reservoirs (triangles), streams/channels (circles) or rice fields (squares). The dominance of host snails (number of transmitting planorbid snails / total number of individuals per site) is represented by the shade of the icon. Maps created using DIVA-GIS 7.5.0. https://diva-gis.org/.

3.3.1 Pesticide tolerance of *Schistosoma* host snails

Among all macroinvertebrates tested, host snails of human-pathogenic schistosomes showed the highest tolerance to the neonicotinoid insecticide imidacloprid and to the organophosphorus insecticide diazinon (Fig. 3.2a and 3.2b; Table 3.1), both of which were commonly found at the study sites. The acute median lethal concentration of imidacloprid after exposure for 24 h (LC_{5024h}, concentration that killed 50 % of test organisms) ranged from 0.007 mg/L for corixidae (true bugs) to 599 mg/L for the non-host snail *Melanoides sp*. The mortality of the host snails *Bulinus africanus* and *Biomphalaria pfeifferi* remained below 10 % even at the highest concentration tested (165 mg/L) so that I could only estimate their respective LC_{50}. This test concentration was close to the solubility limit of imidacloprid in water (610 mg/L (Lewis, 2019)), indicating very high insecticide tolerance of the host snails. The LC_{5024h} of diazinon for other taxa ranged from 0.5 µg/L for baetidae and caenidae

(mayflies) to 13.5 mg/L for the non-host snail *Ceratophallus sp*. Again, the host snails *B. africanus* (15.2 mg/L) and *B. pfeifferi* (33.0 mg/L) were the most tolerant species.

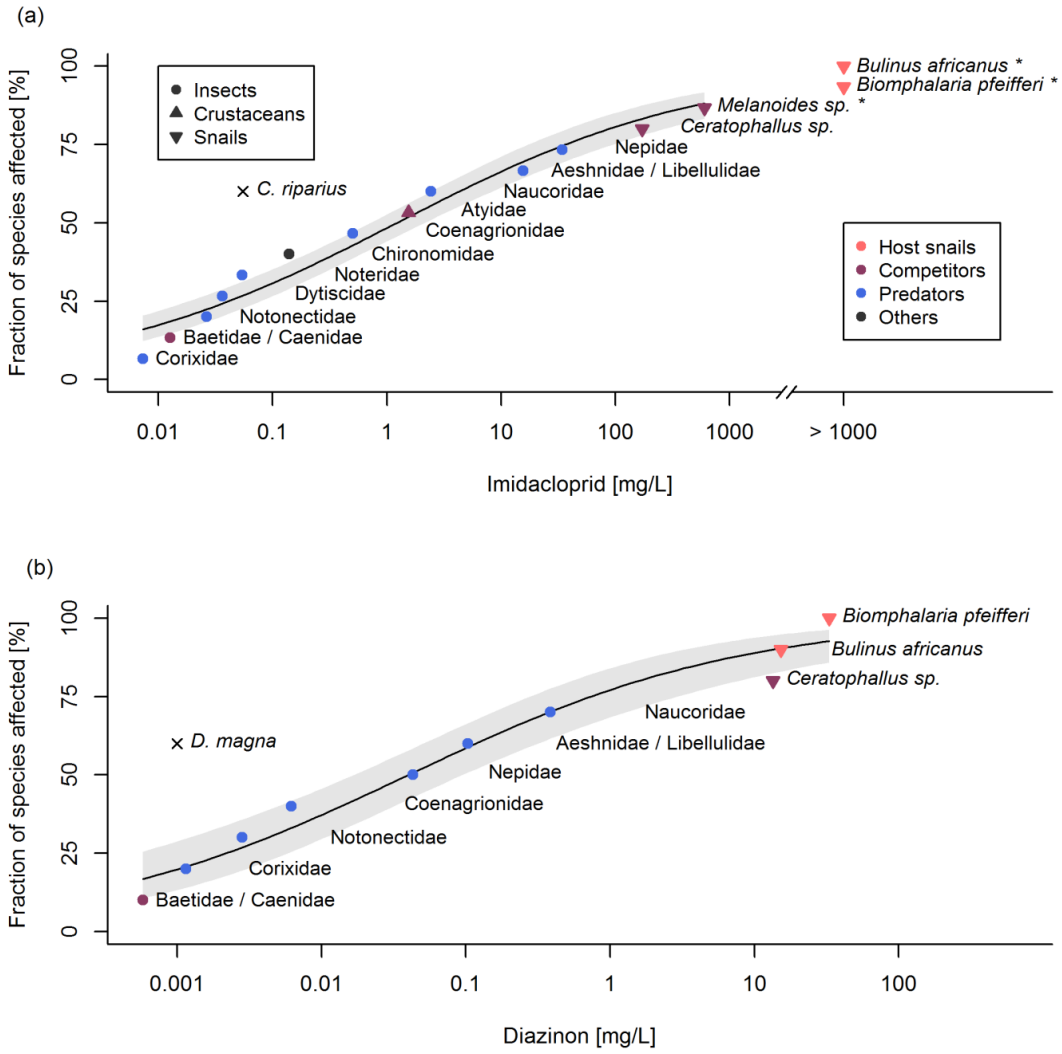


Figure 3.2: Species sensitivity distribution (SSD) of freshwater macroinvertebrates from the study region to common agricultural insecticides. Data points show the acute LC_{5024h} for various species. The SSD curves were fitted using a quasibinomial GLM with logit-link; means ± 95 % confidence intervals are shown. (a) Sensitivity distribution to the neonicotinoid insecticide imidacloprid. $\chi^2 = 230.69$, res. df = 11, $p < 0.001$, McKelvey-Zavoina's pseudo-$R^2 = 0.29$. For *Melanoides sp.*, *Bulinus africanus* and *Biomphalaria pfeifferi* the LC_{50}exceeded the highest test concentration and was extrapolated from non-linear regression (*Melanoides sp.*) or estimated. (b) Sensitivity distribution to the organophosphorus insecticide Diazinon. $\chi^2 = 115.89$, res. df = 8, $p < 0.001$, McKelvey-Zavoina's pseudo-$R^2 = 0.40$. (a, b) The acute LC_{5048h} of the most sensitive standard reference taxa (*Chironomus riparius* and *Daphnia*

magna) was added for comparison (Lewis *et al.*, 2019; USEPA, 2019) and used for the calculation of toxic units (see text).

Table 3.1: LC50s of all taxa investigated in acute toxicity tests using Imidacloprid and Diazinon.

Pesticide	Taxon	LC50 (mg/L)	ln(LC50)	Fraction
Imidacloprid	Micronecta sp. (Corixidae)	0.007	-4.921	0.067
Imidacloprid	Baetidae / Caenidae	0.013	-4.368	0.133
Imidacloprid	Anisops sp. (Notonectidae)	0.026	-3.638	0.200
Imidacloprid	Laccophilus sp. (Dytiscidae)	0.036	-3.316	0.267
Imidacloprid	Noteridae	0.054	-2.921	0.333
Imidacloprid	Chironomidae	0.139	-1.970	0.400
Imidacloprid	Pseudagrion sp. (Coenagrionidae)	0.505	-0.683	0.467
Imidacloprid	Cardina nilotica (Atyidae)	1.548	0.437	0.533
Imidacloprid	Macrocoris sp. / Laccocoris sp. (Naucoridae)	2.429	0.888	0.600
Imidacloprid	Aeshnidae / Libellulidae	15.618	2.748	0.667
Imidacloprid	Ranatra sp. (Nepidae)	34.368	3.537	0.733
Imidacloprid	Ceratophallus sp. (Planorbidae)	171.713	5.146	0.800
Imidacloprid	Melanoides sp. (Thiaridae)	602.890	6.402	0.867
Imidacloprid	Bulinus africanus (Planorbidae)	10000.000	9.210	0.933
Imidacloprid	*Biomphalaria* pfeifferi (Planorbidae)	10000.000	9.210	1.000
Diazinon	Baetidae / Caenidae	0.001	-7.458	0.100
Diazinon	Micronecta sp. (Corixidae)	0.001	-6.772	0.200
Diazinon	Anisops sp. (Notonectidae)	0.003	-5.871	0.300
Diazinon	Pseudagrion sp. (Coenagrionidae)	0.006	-5.088	0.400
Diazinon	Ranatra sp. (Nepidae)	0.043	-3.147	0.500
Diazinon	Aeshnidae / Libellulidae	0.103	-2.268	0.600
Diazinon	Macrocoris sp. / Laccocoris sp. (Naucoridae)	0.385	-0.954	0.700
Diazinon	Ceratophallus sp. (Planorbidae)	13.465	2.600	0.800
Diazinon	Bulinus africanus (Planorbidae)	15.211	2.722	0.900

Diazinon	*Biomphalaria* pfeifferi (Planorbidae)	32.983	3.496	1.000

3.3.2 Pesticide pollution in the study area

The surveyed aquatic habitats showed considerable agricultural pesticide pollution. I analyzed 28 commonly applied active substances and degradation products and detected all the compounds in water samples, ranging from 5 to 27 (median = 20) substances per site. To quantify the toxicity, pesticide concentrations were converted to toxic units (TU) using the formula with *Concentration* being the measured concentration of a pesticide and $LC_{50reference}$ being the LC_{50} of that pesticide for a standard reference organism (typically *Daphnia magna*, Table 3.2) (Tomlin, 2000). Toxic units of -1 or -2 represent pesticide concentrations of 1/10 or 1/100 of the LC_{50}, respectively. Toxic units of pesticides in agricultural European, Siberian and Australian streams typically reach from ≤ -5 to -1 (Becker & Liess, 2017; Schaefer, 2012).

In two sites all pesticides detected were below the limit of quantification such that the toxic unit of the most toxic compound (TU_{max}) could not be calculated. However, in the other sites, the TU_{max} ranged from -6.4 to -1.2 with a median TU_{max} of -2.3 (Table 3.3). The most toxic substances found were the organophosphorous and carbamate insecticides bendiocarb (most toxic substance at 17 sites, TU up to -1.66, Table 3.4), diazinon (most toxic at 15 sites, TU up to -1.71) and pirimiphos-methyl (most toxic at 5 sites, TU up to -1.21). Due to difficulties in the quantification of very low pesticide concentrations, the minimum TU_{max} was set to -5 for further analyses, a threshold at which typically no ecological effects have been observed in field studies (Becker & Liess, 2017; Knillmann, 2018; Schaefer, 2012).

Table 3.2: Pesticides analysed in the water samples. The acute LC_{50}values for *Daphnia magna*, *Chironomus sp.*, *Chironomus riparius* and *Hyalella azteca* were obtained from the PPDB and the ECOTOX data base (Lewis *et al.,* 2019; Brown, 2002). In the PPDB data base LC_{50} values for 48 h exposure on *Chironomus* and *D. magna* were compared and the lowest value was used. If no values were available or if the tolerance of *D. magna* was exceptionally high (neonicotinoids), LC_{50}values for the same species or *H. azteca* were searched in the ECOTOX data base and the median value was used.

Name	CAS Nr.	Application	Class	LC_{50} [µg/L]	Test species	Duration	Source
2,4-Dichlorophenox. Acid	94-75-7	Herbicide	Auxine	11,020.00	*C. sp.*	48 h	ECOTOX
Acetamiprid	135410-20-7	Insecticide	Neonicotinoid	11.56	*C. riparius*	96 h	PPDB
Ametryn	834-12-8	Herbicide	Triazine	28,000.00	*D. magna*	48 h	PPDB
Atrazine	1912-24-9	Herbicide	Triazine	85,000.00	*D. magna*	48 h	PPDB
Azoxystrobin	131860-33-8	Fungicide	Strobilurine	230.00	*D. magna*	48 h	PPDB
Bendiocarb	22781-23-3	Insecticide	Carbamate	30.00	*D. magna*	48 h	PPDB
Carbendazim	10605-21-7	Fungicide	Carbamate	150.00	*D. magna*	48 h	PPDB
Chlormequat	999-81-5	Herbicide	Growth regulator	31,700.00	*D. magna*	48 h	PPDB
Chlorothalonil-4-hydroxy	28343-61-5	Fungicide	Chloronitrile	76,000.00	*C. riparius*	48 h	PPDB
Desethylatrazine	6190-65-4	Metabolite	Triazine	5,100.00	*H. azteca*	96 h	ECOTOX
Desisopropylatrazine	1007-28-9	Metabolite	Triazine	7,200.00	*H. azteca*	96 h	ECOTOX
Diazinon	333-41-5	Insecticide	Organophosphate	1.00	*D. magna*	48 h	PPDB

Diethyltoluamid (DEET)	134-62-3	Repellent	Methylbenzamide	75,000.00	*D. magna*	48 h	PPDB
Diuron	330-54-1	Herbicide	Phenylurea	5,700.00	*D. magna*	48 h	PPDB
Dodemorph	1593-77-7	Fungicide	Morpholine	3,340.00	*D. magna*	48 h	PPDB
Ethofumesate	26225-79-6	Herbicide	Benzofuran	13,520.00	*D. magna*	48 h	PPDB
Fenuron	101-42-8	Herbicide	Acetylurea	502,000.00	*D. magna*	48 h	PPDB
Hexazinone	51235-04-2	Herbicide	Triazine	85,000.00	*D. magna*	48 h	PPDB
Icaridin	119515-38-7	Repellent		100,000.00	*D. magna*	48 h	PPDB
Imidacloprid	138261-41-3	Insecticide	Neonicotinoid	55.00	*C. sp.*	96 h	PPDB
MCPA	94-74-6	Herbicide	Auxine	190,000.00	*D. magna*	48 h	PPDB
Mepiquat	15302-91-7	Herbicide	Piperidine	68,500.00	*D. magna*	48 h	PPDB
Metalaxyl	57837-19-1	Fungicide	Acetylalanine	3,470	*D. magna*	48 h	PPDB
Metolachlor	51218-45-2	Herbicide	Chloroacetanilide	23,500.00	*D. magna*	48 h	PPDB
Metribuzin	21087-64-9	Herbicide	Triazinone	49,000.00	*D. magna*	48 h	PPDB
Pirimiphos-methyl	29232-93-7	Insecticide	Organophosphate	0.21	*D. magna*	48 h	PPDB
Simazine	122-34-9	Herbicide	Triazine	1,100.00	*D. magna*	48 h	PPDB
Terbuthylazine	5915-41-3	Herbicide	Chlorotriazine	21,200.00	*D. magna*	48 h	PPDB

Table 3.3: Pesticide pollution in water samples of the study sites. For each site, the maximum (TU_{max}) and the summed up (TU_{sum}) toxic unit of all analyzed substances is shown, together with the number of substances that could be detected and quantified. At sites 37 and 48 concentrations were too low to for quantification so that no TU_{max} and TU_{sum} could be calculated.

Study site	Coordinates	TU_{max}	TU_{sum}	Most toxic substance	Nr. Detected	Nr. Quantified
1	0°34'43.43"S; 34°36'35.40"E	-3.17	-3.17	Bendiocarb	14	6
2	0°34'53.96"S; 34°32'0.10"E	-4.44	-4.36	Simazine	15	4
3	0°28'29.67"S; 34°32'56.95"E	-2.04	-1.89	Bendiocarb	22	10
4	0°22'48.01"S; 34°38'30.92"E	-1.66	-1.65	Bendiocarb	16	6
5	0°40'50.77"S; 34°32'39.07"E	-2.30	-2.09	Bendiocarb	24	9
6	0°49'46.82"S; 34°23'44.12"E	-2.59	-2.39	Diazinon	17	8
7	0°8'17.02"S; 34°56'8.82"E	-2.70	-2.63	Diazinon	23	12
8	0°8'38.67"S; 34°58'20.86"E	-1.86	-1.85	Pirimiphos-meth.	24	12
9	0°10'18.07"S; 34°54'28.44"E	-1.86	-1.63	Pirimiphos-meth.	28	16
10	0°48'19.63"S; 34°41'54.93"E	-1.21	-1.06	Pirimiphos-meth.	27	17
11	0°22'38.69"S; 34°38'6.98"E	-1.90	-1.89	Bendiocarb	20	10
12	0°23'17.85"S; 34°38'30.02"E	-2.38	-2.36	Bendiocarb	21	10
13	0°33'40.22"S; 34°18'9.70"E	-3.85	-3.85	Bendiocarb	16	4
14	0°29'9.67"S; 34°31'2.85"E	-2.14	-2.11	Carbendazim	12	6
15	0°28'34.93"S; 34°32'41.36"E	-2.00	-1.98	Bendiocarb	28	10
16	0°27'5.24"S; 35°7'15.27"E	-2.60	-2.59	Bendiocarb	18	4
17	0°9'0.91"S; 34°55'49.30"E	-2.58	-2.24	Carbendazim	18	9
18	0°6'46.67"S; 34°47'29.77"E	-2.54	-2.53	Diazinon	15	4
19	0°28'38.92"S; 34°32'38.87"E	-2.25	-2.09	Bendiocarb	17	8
20	0°48'2.29"S; 34°43'45.10"E	-2.34	-2.34	Diazinon	14	1

21	0°32'20.58"S; 35°2'0.91"E	-2.21	-1.92	Diazinon	16	4
22	0°27'45.4"S; 34°33'55.1"E	-2.26	-2.25	Diazinon	20	9
23	0°59'50.59"S; 34°16'55.57"E	-2.36	-2.34	Diazinon	15	7
24	0°19'20.39"S; 34°47'20.33"E	-3.07	-3.06	Bendiocarb	13	4
25	0°53'54.15"S; 34°31'24.54"E	-2.46	-2.39	Diazinon	23	11
26	0°54'29.02"S; 34°33'28.89"E	-1.79	-1.67	Pirimiphos-meth.	18	10
27	0°28'34.33"S; 34°31'56.51"E	-2.07	-1.83	Pirimiphos-meth.	21	12
28	1°3'53.69"S; 34°28'5.52"E	-2.33	-2.30	Diazinon	15	6
29	0°48'31.95"S; 34°43'58.93"E	-2.17	-2.10	Diazinon	19	9
30	0°53'8.15"S; 34°31'20.35"E	-2.29	-2.27	Bendiocarb	14	4
31	0°27'34.68"S; 34°35'40.87"E	-3.50	-3.43	Carbendazim	14	5
32	1°1'18.24"S; 34°37'27.29"E	-2.40	-2.39	Diazinon	17	4
33	0°49'50.42"S; 34°44'44.98"E	-4.09	-3.88	Imidacloprid	19	8
34	0°49'4.79"S; 34°23'34.97"E	-2.55	-2.45	Bendiocarb	21	10
35	0°27'47.58"S; 34°32'55.31"E	-1.89	-1.88	Bendiocarb	20	10
36	0°46'39.89"S; 34°12'21.19"E	-6.44	-6.34	Diethyltoluamid	4	2
37	0°48'47.38"S; 34°13'15.06"E	/	/	Ametryn	6	0
38	0°39'27.61"S; 34°42'37.10"E	-2.26	-2.06	Bendiocarb	16	8
39	0°39'24.17"S; 34°41'57.84"E	-2.06	-1.98	Diazinon	17	10
40	0°30'48.53"S; 34°17'29.08"E	-5.81	-5.80	Simazine	8	2
41	0°23'35.22"S; 35° 0'35.88"E	-4.94	-4.94	Simazine	8	1
42	0°59'40.04"S; 34°17'26.47"E	-3.66	-3.66	Bendiocarb	9	2
43	0°59'40.04"S; 34°17'26.47"E	-1.81	-1.76	Diazinon	23	15
44	0°59'9.30"S; 34°35'5.25"E	-2.54	-2.53	Bendiocarb	20	10
45	0°27'5.46"S; 35°13'8.39"E	-3.93	-3.81	Imidacloprid	10	3

46	0°29'12.72"S; 35°10'58.85"E		-2.59	-2.24	Diazinon	17	9
47	0°30'54.82"S; 35° 4'49.80"E		-2.24	-2.23	Diazinon	12	3
48	0°19'1.31"S; 35°0'22.66"E		/	/	Acetamiprid	1	0

Table 3.4 Ranking of the analysed pesticides according to the environmental toxicity observed. The substances were ordered according to the maximum toxic unit (Max. TU) observed in water samples from all study sites. Additionally, the number of sites is reported at which a substance showed the highest toxic unit among all pesticides analyzed.

Pesticide	CAS Nr.	Application	Class	Max. TU	Most toxic at nr. Of sites	Detected at nr. Of sites
Pirimiphos-methyl	29232-93-7	Insecticide	Organophosphate	-1.21	5	6
Bendiocarb	22781-23-3	Insecticide	Carbamate	-1.66	17	31
Diazinon	333-41-5	Insecticide	Organophosphate	-1.71	15	36
Carbendazim	10605-21-7	Insecticide	Carbamate	-2.14	3	46
Acetamiprid	135410-20-7	Insecticide	Neonicotinoid	-2.80	0	39
Imidacloprid	138261-41-3	Insecticide	Neonicotinoid	-3.24	0	16
Simazine	122-34-9	Herbicide	Triazine	-3.83	3	46
2,4-Dichlorophenox		Herbicide	Auxine			48
acetic acid	94-75-7			-4.32	0	
Metalaxyl	57837-19-1	Fungicide	Acetylalanine	-4.40	0	47
Dodemorph	1593-77-7	Fungicide	Morpholine	-4.42	0	40

Ametryn	834-12-8	Herbicide	Triazine	-4.68	0	6
Hexazinone	51235-04-2	Herbicide	Triazine	-4.74	0	15
Atrazine	1912-24-9	Herbicide	Triazine	-4.88	0	45
Azoxystrobin	131860-33-8	Fungicide	Strobilurine	-4.98	0	32
Ethofumesate	26225-79-6	Herbicide	Benzofuran	-5.09	0	31
Metribuzin	21087-64-9	Herbicide	Triazinone	-5.42	0	8
Desethylatrazine	6190-65-4	Metabolite	Triazine	-5.61	0	47
Metolachlor	51218-45-2	Herbicide	Chloroacetanilide	-5.70	0	27
Diuron	330-54-1	Herbicide	Phenylurea	-5.76	0	15
Desisopropylatrazine	1007-28-9	Metabolite	Triazine	-5.77	0	17
Terbuthylazine	5915-41-3	Herbicide	Chlorotriazine	-5.96	0	31
Mepiquat	15302-91-7	Herbicide	Piperidine	-6.17	0	47
Icaridin	119515-38-7	Repellent		-6.17	0	47
Chlormequat	999-81-5	Herbicide	Growth regulator	-6.43	0	1
Diethyltoluamid (DEET)	134-62-3	Repellent	Methylbenzamide	-6.43	0	48

MCPA	94-74-6	Herbicide	Auxine	-7.15	0	48
Chlorothalonil-4-hydro		Fungicide	Chloronitrile			13
xy	28343-61-5			-7.80	0	
Fenuron	101-42-8	Herbicide	Acetylurea	-8.38	0	47

3.3.3 Environmental factors driving host snail abundance

I investigated the influence of 27 environmental variables on the abundance of host snails, covering habitat type, land use, water chemistry and the composition of the macroinvertebrate community (Table 3.5). Host snails were found in 9 out of a total of 48 sites investigated in 2017; at one site they were infected with human-pathogenic schistosomes. The abundance of host snails encompasses the incidence, i.e., the probability of a population to occur at a given site, and the density of existing populations. Both endpoints can be driven by different environmental factors and thus were analyzed separately in a first step.

When each environmental variable was considered individually, the incidence of host snails increased significantly with pesticide toxicity ($n = 48$, $\chi^2 = 7.71$, res. df = 46, $p = 0.005$, Fig. 3.3), species diversity ($\chi^2 = 4.42$, res. df = 46, $p = 0.035$) and species richness ($\chi^2 = 4.39$, res. df= 046, $p = 0.036$). Additionally, the incidence of host snails decreased with increasing dissolved oxygen ($\chi^2 = 8.06$, res. df = 46, $p = 0.004$) and with the increasing dominance (proportion on all macroinvertebrates) of other grazers and herbivores that act as potential competitor species ($\chi^2 = 9.09$, res. df = 46, $p = 0.003$; Tab. 3.4).

Table 3.5: Minimal adequate model for environmental effects on the abundance of *Schistosoma* host snails. I selected all environmental variables that on their own showed a significant effect on the incidence or on the population density of host snails and combined them in an additive hurdle model. Using backward elimination based on likelihood ratio tests, non-significant environmental variables (species diversity) were removed. Because data have been standardized, importance of the environmental variables on the incidence or on the population density of snails can be compared within each part of the model based on their regression coefficients; coefficients far from zero indicate high (positive or negative) impact. Log-likelihood = -42.43 on 8 df and 40 res. df; McFadden's pseudo-$R^2 = 0.28$.

Term	Coefficient	Std. error	*z*	*p*	
Count part (zero-truncated negative binomial with log-link; models population density)					
Intercept	1.92	0.60	4.58	0.001	**

Turbidity	-3.12	1.17	-2.68	0.007	**
ln(Distribution coefficient)	-0.58	0.79	-0.74	0.461	
Zero part (binomial with complementary log-log-link; models incidence)					
Intercept	-5.11	1.90	-2.69	0.007	**
Pesticide pollution	2.73	1.29	2.12	0.034	*
Dominance competitors	-2.30	1.09	-2.12	0.034	*
Species richness	1.81	0.92	1.97	0.048	*
Dissolved oxygen	-0.92	0.45	-2.02	0.044	*

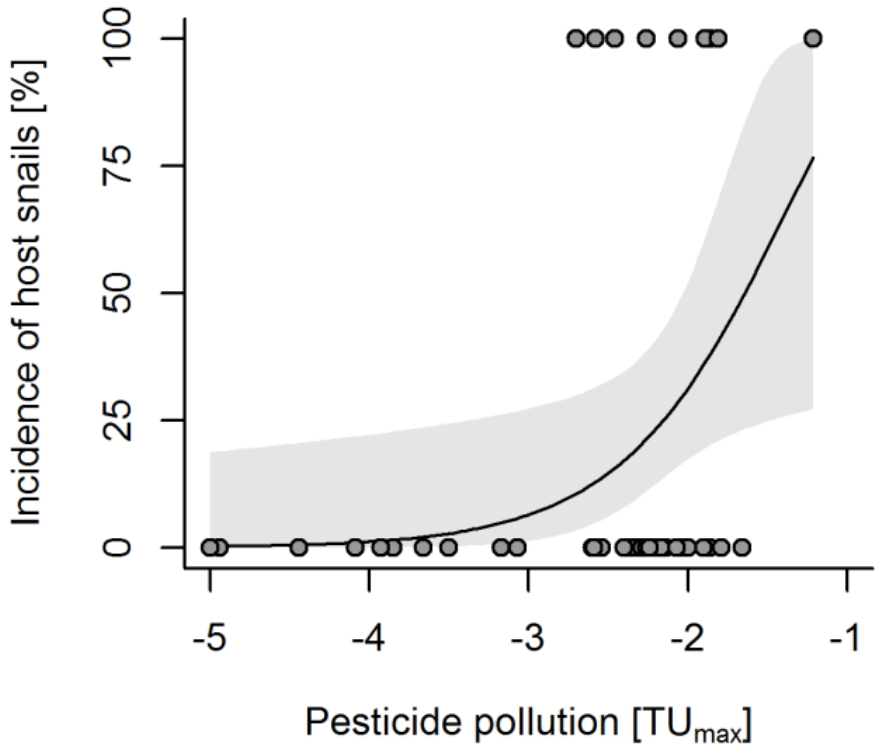


Figure 3.3: Pesticide pollution increases the incidence (probability of occurrence) of snails that act as hosts of schistosomiasis. Binomial GLM with complementary log-log link function; $\chi^2 = 7.60$, res. df = 46, $p = 0.006$, McFadden's pseudo-$R^2 = 0.16$. Means ± 95 % confidence intervals are shown. Pesticide pollution was quantified as $\log_{10}$ of the maximum

ratio of a pesticide concentration measured in a grab sample of water vs. the acute $LC_{50}$of that pesticide for a standard reference organism (TU_{max}). TU_{max} of marginally polluted sites ($n = 4$) was set to a minimum of TU -5.

Environmental effects on the density of host snail populations were driven by a single stream (Tab. 3.5). This stream was characterized by extraordinarily high numbers of host snails and other macroinvertebrates. The site was located 100 m downstream of a bathing and washing area and was the only site at which infected host snails were found. When I excluded this site as an outlier, population density was explained only by a decrease in density with increasing turbidity ($n = 8$, $\chi^2 = 4.50$, res. df = 6, $p = 0.034$).

Table 3.6 Environmental effects on the population density of schistosomiasis hosts in surface waters of the study area. Each environmental variable was fitted using a one-way GLM with a zero-truncated negative-binomial distribution and log-link. In the upper part of the table results are reported with all study sites considered where hosts snails had been found; $n = 9$ and res. Df = 7 in all models. Only those environmental variables are presented that showed a (marginally) significant effect ($p < 0.1$); see Tab. S4 for a complete list of environmental variables tested and for the units of measurement. Below, effects of the same variables are shown when site 39 (with extraordinary mass development of host snails) was excluded as a highly influential outlier; here $n = 8$ and res. Df = 6 in all reported models. Model coefficients are reported together with their standard error and McFadden's pseudo-R^2. χ^2 and p values are reported from a likelihood ratio test against the null model without explanatory variables

Environmental variable	Intercept	Slope	Pseudo-R^2	X^2	p
For all study sites					
Depth	**11.66 ± 3.23**	**-2.26 ± 0.81**	**0.09**	**6.25**	**0.012***
Acidity	10.37 ± 1.99	-1.09 ± 0.28	0.14	10.41	0.001**
Turbidity	12.60 ± 2.95	-2.01 ± 0.60	0.07	5.00	0.025*
PO_4	4.75 ± 1.09	-0.13 ± 0.06	0.04	2.79	0.095
Species richness	-5.98 ± 3.74	3.52 ± 1.49	0.06	4.47	0.034*
Evenness	5.96 ± 1.33	-6.23 ± 2.41	0.06	4.02	0.045*
Macroinvertebrate abundance	-3.92 ± 2.36	1.43 ± 0.50	0.11	7.90	0.005**
Dominance predators	5.49 ± 0.84	-4.39 ± 1.21	0.13	9.08	0.003**
Dominance competitors	1.52 ± 0.59	6.94 ± 2.68	0.09	6.22	0.013*
Without site 39					

Depth	**2.93 ± 3.39**	**-0.19 ± 0.82**	**< 0.01**	**0.03**	**0.871**
Acidity	7.55 ± 6.72	-0.72 ± 0.90	0.01	0.72	0.395
Turbidity	5.73 ± 1.43	-0.73 ± 0.29	0.09	4.50	0.034*
PO_4	2.83 ± 0.58	-0.04 ± 0.03	0.03	1.73	0.188
Species richness	-0.80 ± 2.54	1.22 ± 1.04	0.02	1.16	0.281
Evenness	3.21 ± 0.86	-1.94 ± 1.50	0.02	1.07	0.302
Macroinvertebrate abundance	-0.83 ± 1.83	0.68 ± 0.41	0.04	2.27	0.132
Dominance predators	3.65 ± 1.02	-2.10 ± 1.37	0.05	2.33	0.127
Dominance competitors	2.16 ± 0.51	0.02 ± 3.17	< 0.01	< 0.01	0.996

In a second step, I combined the effects identified on the incidence and population density in a hurdle model in order to rank the relevance of the environmental variables in explaining the overall abundance of host snails. Stepwise regression identified that the incidence of host snails increased primarily with pesticide pollution, followed by an increase with the decreasing dominance of potential competitors, with increasing species richness and with a decreasing amount of dissolved oxygen. The density of host snail populations only decreased with turbidity.

Table 3.7 Environmental effects on the incidence of schistosomiasis hosts in surface waters of the study area. Each environmental variable was fitted using a one-way binomial GLM with cloglog-link. The unit and the transformation of each environmental variable prior to analysis is given in squared brackets. For numerical variables, model coefficients are reported together with their standard error; $n = 48$ for each numeric model. For categorical variables, the back-transformed mean of each factor level is reported together with 95 % confidence intervals and the number of observations. X^2 and p are reported from a likelihood ratio test against the null model without explanatory variables.

Numerical variable	Intercept	Slope	Res. Df	X^2	p
Flow velocity [ln(m/s)]	-1.64 ± 0.63	-0.02 ± 0.17	46	0.02	0.900
Depth [ln(cm)]	-3.81 ± 2.65	0.57 ± 0.65	46	0.84	0.359
Temperature [°C]	-1.50 ± 1.87	< -0.01 ± 0.07	46	< 0.01	0.969
Conductivity [ln(µS/cm)]	-5.47 ± 3.07	0.79 ± 0.60	46	1.90	0.170
Acidity [pH]	1.65 ± 2.85	-0.44 ± 0.39	46	1.02	0.312
Dissolved oxygen [ln(mg/L)]	0.47 ± 0.70	-1.60 ± 0.58	46	8.06	0.004**
Turbidity [ln(NTU)]	-0.92 ± 1.07	-0.13 ± 0.20	46	0.41	0.520
Carbonate hardness [ln(°dH)]	-3.03 ± 1.16	0.89 ± 0.63	46	2.25	0.134
NH_4 [mg/L]	-1.51 ± 0.38	-0.88 ± 2.69	46	0.11	0.746
PO_4 [mg/L]	-1.24 ± 0.73	-0.02 ± 0.04	46	0.23	0.629
NO_{3-} [mg/L]	-1.13 ± 0.45	-0.06 ± 0.05	46	1.59	0.207
NO_{2-} [ln(mg/L)]	-0.72 ± 1.16	0.26 ± 0.35	46	0.54	0.463

Species richness) [ln(*n* taxa)]	-5.29 ± 2.11	1.62 ± 0.86	46	4.39	0.036*
Evenness [J']	-2.07 ± 1.20	0.93 ± 2.14	46	0.16	0.688
Species diversity [H']	-4.21 ± 1.53	1.64 ± 0.84	46	4.42	0.035*
SASS5 [ln(ASPT)]	2.12 ± 3.11	-2.33 ± 1.99	46	1.56	0.211
Macroinv. Abund. [ln(*n* ind.)]	-2.47 ± 2.16	0.20 ± 0.48	46	0.18	0.672
Dominance predators [%]	-2.05 ± 0.95	0.73 ± 1.31	46	0.33	0.564
Dominance competitors [%]	-0.36 ± 0.45	-4.58 ± 1.87	46	9.09	0.003**
Pesticide pollution [TU_{max}]	2.46 ± 1.69	1.72 ± 0.79	46	7.60	0.006**
Emerged vegetation cover [%]	-1.37 ± 0.36	-1.73 ± 1.84	46	1.33	0.249
Floating vegetation cover [%]	-1.61 ± 0.35	0.70 ± 1.91	46	0.13	0.716
Submerged vegetation [%]	-1.69 ± 0.36	5.65 ± 3.95	46	1.26	0.262
Detritus cover [%]	-1.57 ± 0.36	-0.03 ± 1.68	46	< 0.01	0.984

Categorical variable	Mean (95 % CI)	*n*	Res. Df	X^2	*p*
Habitat: Main tributary	0.11 (0.03 – 0.38)	18	42	6.31	0.277
Minor tributary	0.40 (0.17 – 0.74)	10			
Irrigation channel	0.20 (0.03 – 0.79)	5			
Oxbow lake	< 0.01 (0.00 – 1.00)	3			
Reservoir	0.25 (0.07 – 0.68)	8			
Rice field	< 0.01 (0.00 – 1.00)	4			
Land use: Natural	0.30 (0.11 – 0.67)	10	44	6.37	0.095
Agricultural	0.16 (0.07 – 0.34)	32			
Semi-urban	< 0.01 (0.00 – 1.00)	5			
Industrial	1 (0.00 – 1.00)	1			
Farm type: Natural	0.27 (0 .10– 0.63)	11	43	1.37	0.850
Subsistence	0.21 (0.07 – 0.53)	14			
Agroforestry	< 0.01 (0.00 – 1.00)	1			
Commercial	0.13 (0.04 – 0.44)	15			
Irrigation scheme	0.14 (0.02 – 0.67)	7			
Crop type: Maize	0.15 (0.04 – 0.49)	13	35	3.93	0.415
Rice	0.09 (0.01 – 0.49)	11			
Sugar cane	0.42 (0.16 – 0.83)	7			
Tea	0.14 (0.02 – 0.67)	7			
Other	< 0.01 (0.00 – 1.00)	2			

Results from stepwise regression are sensitive to the method used for model selection. Therefore, I additionally applied a multi-model approach by subjecting the full hurdle model to hierarchical partitioning (Fig. 3.4). Here the abundance of host snails was most strongly affected by the dominance of potential competitors, followed by the effect of pesticide pollution. In accordance with the results from backward selection, turbidity, species richness

and dissolved oxygen showed intermediate effects, and species diversity was least important again.

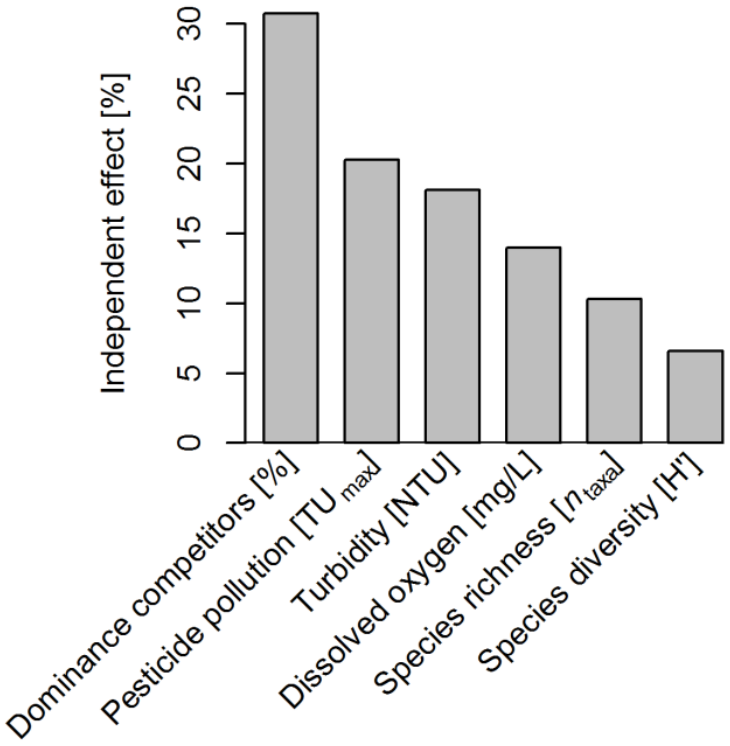
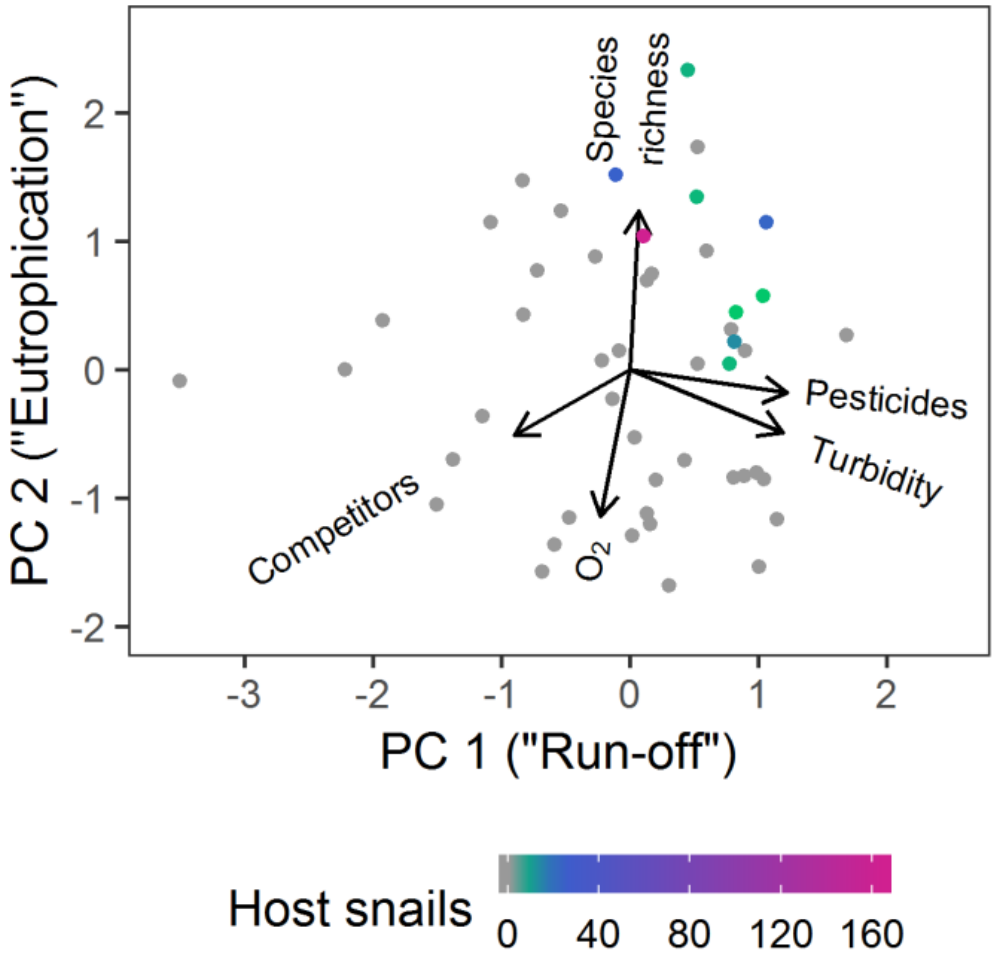


Figure 3.4: Ranking the relevance of environmental variables in driving the abundance of host snails. I combined all environmental variables that on their own showed a significant effect on the incidence or on the population density of host snails and combined them in a hurdle model. The model was subjected to hierarchical partitioning to identify the independent contribution of each environmental variable to the goodness-of-fit (quantified as log-likelihood of the hurdle model). Each step, an environmental variable was either included both in the zero and the count part of the model or excluded completely.

In the next step, I confirmed the main drivers that potentially underlie the identified environmental variables using a principal component analysis (PCA). The first principal component explained 33.0 % of the total variation among the sites and was associated with typical effects of surface run-off after heavy rainfall (Fig. 3.5): It increased with pesticide pollution and turbidity and with decreasing dominance of potential competitor species of the host snails. The second principal component additionally explained 29.4 % of the variation



and increased with species richness, with a decreasing amount of dissolved oxygen (indicating increasing oxygen consumption) and with decreasing dominance of potential competitors. Thus, the second principal component likely reflected an increase of host snails with eutrophication that supports more taxa but results in oxygen depletion. Moreover, the second principal component increased with the overall number of macroinvertebrate individuals as an indicator of productivity (n = 48, F = 8.12, res. df = 46, p = 0.007, R^2 = 0.15) which further supported its interpretation as eutrophication. Host snails were only found

when both the effects of run-off and eutrophication were high, which resulted in a decreased dominance of potential competitors (Fig. 3.5).

Figure 3.5: Principal component analysis of the environmental variables that drive the abundance of *Schistosoma* hosts. The 1st principal component explains 33.0 % of the variation and is associated with pesticide pollution, turbidity and the dominance of potential competitor species of the host snails. The 2nd principal component explains 29.4 % of the variation and is associated with the species richness, dissolved oxygen and again with the dominance of competitors. Colors indicate the number of host snails collected.

3.3.4 Ecological mechanisms

To better understand the ecological mechanisms through which pesticides affect host snails, I investigated effects of pesticides on the macroinvertebrate community composition. Pesticide pollution affected neither the dominance of all grazers (host snails and their potential competitors; $n = 48$, $\chi^2 = 0.41$, res. df = 46, $p = 0.520$) nor of predators ($\chi^2 = 0.37$, res. df = 46, $p = 0.541$) or other macroinvertebrates ($\chi^2 = 2.63$, res. df = 46, $p = 0.105$). Thus, the overall distribution of grazers, predators and other taxa within the community did not significantly change with pesticide pollution (Fig. 3.6a). However, within the guild of grazers, pesticide pollution increased the dominance of snails (Fig. 3.6b) which were much more tolerant to pesticides than their highly sensitive insect competitors (Fig. 3.2). In contrast, pesticide pollution did not affect the composition of predatory macroinvertebrates (PERMANOVA; $n = 48$, $F = 0.78$, res. df = 46, $p = 0.679$) which generally showed intermediate sensitivity to pesticides (Fig. 3.2). Additionally, the taxonomic composition of potential predators had no effect on the balance of snails vs. potential competitors: The first principal component of a PCA on the composition of predators did not explain the dominance of snails within the grazers ($n = 47$, $\chi^2 = 0.02$, res. df = 45, $p = 0.890$); the same was observed for higher principal components. Therefore, I conclude that pesticides indirectly favored host snails through negative effects on their competitors but not on their predators.

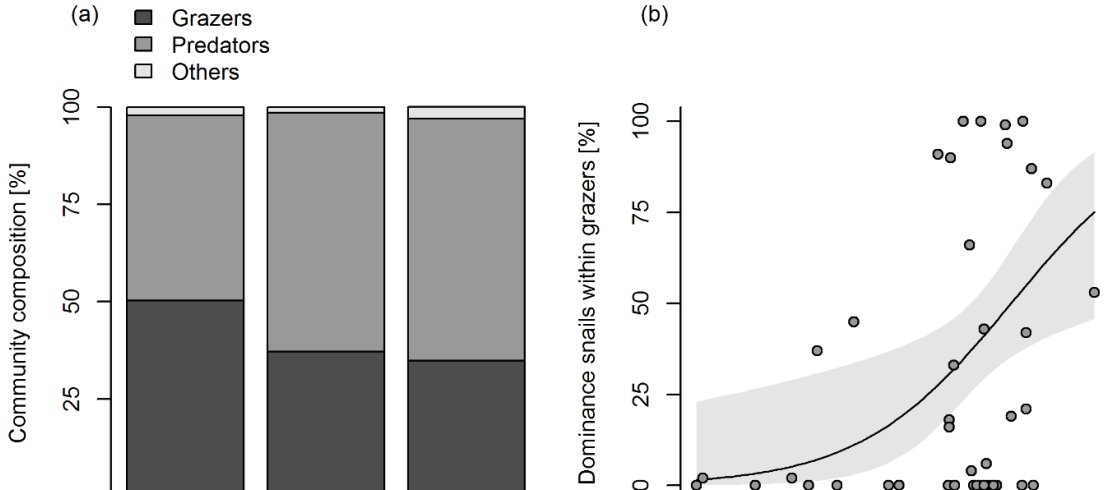


Figure 3.6: Pesticide pollution favors tolerant snails over less tolerant competitors. (a) No significant change in the community composition of grazers, predators and other taxa with pesticide pollution (PERMANOVA; $n = 48$, $F = 0.80$, res. df = 46, $p = 0.502$, $R^2 = 0.02$). For the graph, the range of TU_{max} values was evenly split in three categories, and for each pollution category the mean proportion of each guild on the macroinvertebrate community is shown. Because some taxa belong to more than one guild, I calculated proportions as the individuals in a guild divided by the summed-up individuals in all guilds (≠ the total individual number) so that the proportions sum up to 1. (b) Within the guild of grazers, the dominance of snails increases with pesticide pollution ($n = 47$, $\chi^2 = 13.82$, res. df = 45 $p < 0.001$, McKelvey-Zavoina's pseudo-$R^2 = 0.37$). One site was omitted because no grazers were found. Quasi-binomial GLM with logitlink function; means ± 95 % confidence intervals are shown.

3.4 Discussion

To our knowledge this is the first field study providing evidence that ecological effects of agricultural pesticides can pose a serious risk to human health. Laboratory studies have already shown how pesticide pollution can increase risk of schistosomiasis (Halstead *et al.*, 2018). I demonstrate a second mechanism with both field and laboratory data showing how such conditions favor the disease by benefiting the host snail. Host snails of schistosomiasis showed highest tolerance to insecticides amongst all tested macroinvertebrate taxa. Host snails were also solely found in habitats that were at least moderately affected by pesticides and eutrophication. In these conditions, snails replaced more sensitive potential competitor species. The study thus sheds light on an important risk factor for the transmission of schistosomiasis that has been largely overlooked in previous research on the ecology of host snails and in public health programs.

3.4.1 Pesticide pollution in the study area

Concentrations of the highest exposure (TU_{max}) ranged from <-5 to -1.21, representing a 10^{th} to < $100{,}000^{th}$ of the acute lethal median concentration for standard test organisms. This range of pesticide exposure is comparable to several previous studies conducted in agricultural streams of Europe and Australia (Becker & Liess, 2017; Beketov, 2013; Liess, 2005). Given the predominance of subsistence farming in the study area, the results illustrate that pesticide pollution in freshwater is not limited to intensified agriculture. Apparently, the risk of pesticide runoff from agricultural fields is high in the study region. During the rainy seasons in April - June and in October - December, heavy rainfalls erode the cleared land, as indicated by the high turbidity observed in streams during sampling. Because the water bodies are typically not protected by riparian strips from surface run-off, pesticides are washed from flooded agricultural fields into the streams and reservoirs (Liess, 1999). In addition, plant protection products are sold in Kenya at comparably low prices without the need for a certificate of competence. This makes pesticides available even for small farmers who may lack enough training and equipment to comply with the proposed environmentally safe use.

The study focused on a broad set of agricultural pesticides typically detected in water. It cannot be excluded that the overall pesticide toxicity might have been even higher due to additional compounds such as pyrethroid insecticides that require different analytical methods. However, pyrethroids typically occur concurrently with the compounds detected and show a similar range of toxicity (Becker & Liess, 2017; Münze *et al.,* 2017). In previous

studies (Becker & Liess, 2017; Beketov, 2013; Liess & von der Ohe, 2005) pesticides had been sampled during the peak exposure following run-off events after heavy rainfalls. Event-triggered sampling was not feasible in our study area. However, I sampled during the main rainy season at which I expected the highest pesticide exposure from run-off. Therefore, the TU_{max} values determined to characterize the toxic pressure in this study are comparable to those of previous investigations.

3.4.2 Effects of pesticides on schistosomiasis infection

Host snails of human-pathogenic schistosomes were found exclusively in freshwaters that were at least moderately polluted with pesticides (TU_{max} >= -3) and at least mesotrophic. Physicochemical and land-use parameters had no significant effect on the abundance of host snails, and additional pollutants such as pharmaceuticals, personal care products and industrial chemicals have been shown to cause considerably lower environmental risk than pesticides at the study sites (Kandie, 2020a). This was observed across various habitats ranging from reservoirs to irrigation channels and streams.

The results support our hypothesis that agricultural pesticide pollution in tropical freshwaters increases the risk of infection with schistosomiasis: Snails as intermediate hosts of human-pathogenic schistosomes are mandatory to close the infection cycle, and humans can become infected only from larval forms (cercariae) released by snails into the water (CDC, 2018). Besides freshwater contamination with infected human excreta and human contact with freshwater infested with cercariae, presence of host snails is a major risk factor for transmission (Agents, 2012). Two human-pathogenic trematodes occur in the study region: *Schistosoma mansoni* parasitizes snails of the genus *Biomphalaria sp.* and causes intestinal schistosomiasis, whereas *S. haematobium* parasitizes certain snails of the *Bulinus africanus* complex and causes urogenital schistosomiasis (King, 2015). Access to sanitation is often insufficient in the densely populated study area (Chadeka, 2019; Odhiambo *et al.*, 2014), and therefore many people are exposed to non-sanitized freshwater during activities such as bathing (particularly school children), washing and field work (IARC, 2012). This is especially pronounced at the shore of Lake Victoria which suffers from a high disease burden (Chadeka, 2019; Mwandawiro, 2019). When traveling, schistosomiasis transmission may be imported to inland areas if host snails are present (WHO, 2008). In these conditions, I expect that the risk of infection is influenced by the occurrence of intermediate host snails.

Our finding of pesticide-induced shifts in the community composition towards more snails are in line with various studies that reported significant ecological effects of pesticides even

in streams with low concentrations resembling a TU_{max} of -2 to -4. These effects include changes in the macroinvertebrate community composition towards more tolerant taxa (Liess, 2005), reduced leaf litter breakdown (Münze, 2017) and the development of pesticide resistance (Becker & Liess, 2017). The results, however, contrast the common perception of the environmental risk of pesticides. For example, according to the European framework for the registration of plant protection products, environmental concentrations < 1 % of the acute LC_{50} of the most sensitive standard reference organism are generally considered safe [45]; this would resemble a TU_{max} up to -2. No such threshold concentrations have been defined in Kenya, but pesticides need to be considered environmentally safe by the national Pest Control Product Board (PCPB) before registration (PCPB-Kenya, 2006). The present study shows that pesticides nevertheless affect the community composition of freshwater macroinvertebrates and that these ecological effects can have serious consequences for human health, hence the need for revision of acceptable regulatory concentrations.

3.4.3 Ecological mechanisms supporting host snails

Given the very high pesticide tolerance of snails compared to the pesticide concentrations measured in the environment, a direct effect of pesticides on the observed host snails appears unlikely. Instead, pesticides may indirectly favor host snails through adverse effects on their antagonistic species such as predators and competitors. A recent mesocosm study showed that herbicides and insecticides can favor host snails of human-pathogenic schistosomes through effects on predators and the support of periphyton as food source for snails (through effects on antagonistic planktonic algae); however, the study did not collect field data, nor did it discuss potential effects on competitor species (Halstead, 2018). The observed effects on predators in the mesocosm study contrast our results, probably because pesticide concentrations were 3 - 4 orders of magnitude higher than those observed at our study sites. Such concentrations may affect even taxa such as predators that showed generally intermediate pesticide tolerance in our tests and in previous studies (Beketov & Liess, 2008; Wogram & Liess, 2001). I observed that with increasing pesticide pollution snails replaced grazing insects that are known to compete with the more tolerant snails (Yeung, 2012) and are generally highly sensitive to insecticides and some fungicides[47,48]. In fact, pesticides have been shown to affect the survival and emergence of aquatic insects at concentrations down to 0.005 % (4 orders of magnitude below) of their acute LC_{50} (Beketov & Liess 2005; Liess & Schulz, 1996). Moreover, the calculation of toxic units for additional trophic levels revealed highest risk for insects and crustaceans compared to algae and vertebrates in our study sites

(Kandie, 2020a). Therefore, I focused on the toxicity of pesticides to invertebrates. Our results indicate that in the field, pesticides favor snails mainly through negative effects on more sensitive competitors. Pesticide pollution was closely related with turbidity, as both factors increase with rainfall induced flooding (Liess & Schulz, 1999; Shen, 2018). Nevertheless, these factors showed contrasting effects on the abundance of host snails (increased incidence vs. decreased population density). I hypothesize that flooding results in a short-term reduction of host snail populations due to increased flow velocity (Woolhouse, 1990), whereas pesticide exposure in the long-term facilitates the establishment of tolerant taxa such as snails (Liess & von der Ohe, 2005; Liess *et al.*, 2013). This may explain the generally low population densities of host snails observed during the rainy season and the more obvious link of pesticide pollution with incidence than with population density.

3.5 Conclusions

The present case study illustrates that serious consequences of agricultural pesticide pollution arises for public health, even at concentrations considered safe within the traditional risk assessment. Given that pesticide application – particularly in developing countries - is predicted to increase 2- to 5-fold from 2000 to 2050 to meet the food demand of a growing human population, freshwater pollution and its ecological effects will aggravate (Alexandratos & Bruinsma, 2012; Tilman *et al.*, 2001). The results underline the urgent need for reassessing the environmental risk of low pesticide concentrations and for integrated disease management that includes a focus on the regulation and management of pesticides in areas where schistosomiasis is endemic or might be introduced due to potentially favorable ecological conditions.

CHAPTER FOUR

HOW PESTICIDES AFFECT THE *SCHISTOSOMA* PARASITES

Abstract

Schistosomiasis is a neglected tropical disease caused by trematodes of the genus *Schistosoma*. The pathogen is transmitted via freshwater snails as intermediate hosts. Exposure to agricultural pesticides may affect the pathogen infectivity and the host snail immunity and thus may increase or decrease the risk of schistosomiasis transmission. In bioassays I tested the tolerance of the free-swimming infective life stages (miracidia and cercariae) of *Schistosoma mansoni* to the insecticides diazinon and imidacloprid. I also tested whether both pesticides decreased the ability of miracidia to infect and further develop as sporocysts within the host snail *Biomphalaria pfeifferi.* Exposure to diazinon and imidacloprid for 12 h immobilized 50% of miracidia at 20.91 ± 1.19 µg/L (EC50±SE) and 15.47 ± 1.74 µg/L, and 50% of cercariae at 14.82 ± 0.39 µg/L and 337.12 ± 67.21 ± 0.50 µg/L, respectively. The infectivity of miracidia decreased at sublethal concentrations of ~20% the 6 hr EC50 of miracidia (10.5 µg/l diazinon, 48.8 µg/L imidacloprid) but was not affected at environmentally relevant concentrations (1.05 µg/L diazinon, 4.88 µg/L imidacloprid). The development of sporocysts was not significantly affected at any test concentration. The insecticide tolerance of *S. mansoni* was considerably lower than those of its intermediate host *B. pfeifferi* and comparable to those of standard freshwater invertebrate test organisms. Recently, pesticides have been shown to foster an increase in the highly tolerant host snails due to indirect effects via the food web. Nevertheless, I showed that pesticides do not affect the interaction of *S. mansoni* with its intermediate host even at concentrations well above those that are environmentally relevant. Freshwater pollution with agricultural pesticides is therefore likely to increase the risk of schistosomiasis transmission; mitigation measures should be considered as part of public health programs.

4.1 Introduction

Schistosomiasis remains a major public health problem in much of the world (WHO, 2020) despite the great effort to eliminate this disease. The cause are parasitic trematodes of the genus *Schistosoma* that use freshwater snails as intermediate hosts (King, 2015). In western Kenya, studied as an example of highly endemic areas, two forms of the disease are present: Intestinal schistosomiasis is caused by *Schistosoma mansoni,* and urinary schistosomiasis is caused by *Schistosoma haematobium* (Brooker *et al.*, 2009). These trematodes parasitize

planorbid snails from the genus *Biomphalaria* in case of *S. mansoni* and from the genus *Bulinus* in case of *S. haematobium* (Gryseels *et al.*, 2006). The trematodes penetrate the snails as free-swimming larvae (miracidia) and undergo asexual reproduction as sporocysts within the snail before maturation into human-infecting free-swimming cercariae. This process takes about four weeks within the snail (King, 2015).

Agricultural activities have been shown to increase the risk of schistosomiasis by creating suitable habitats such as dams and canals for irrigation which support the development of host snails whilst preventing their predators from accessing them (Sokolow *et al.*, 2017). Recent field work has shown that exposure of freshwater to agricultural pesticides can increase the likelihood of finding host snails in potential habitats, as well as the density of existing host snail populations (Becker *et al.*, 2020). Pesticides foster the highly tolerant host snails indirectly by affecting their more sensitive competitors (Becker *et al.*, 2020) and predators (Haggerty *et al.*, 2022; Halstead *et al.*, 2018). In western Kenya, pesticide residues found in freshwater samples and within freshwater snails were most toxic to freshwater arthropods (represented by the test species daphnia magna), followed by fish (represented by Onchorhynchus mykiss) (Kandie *et al.*, 2020b). As several fish species are potential predators of the host snails (Kefi *et al.*, 2012; Lundeba, 2007; Slootweg, 1987), a reduction in their numbers would allow for increase in snails. The toxicity observed for *Daphnia* also means that *Schistosoma* host snails are likely to benefit, as *Daphnia* represent the wider macroinvertebrate community that consists of both potential predators and competitors (Becker *et al.,* 2020; Halstead *et al.* 2018). Consequently, pesticide pollution may increase the risk of schistosomiasis transmission by supporting increased numbers of intermediate host snails. However, assessing effects of pesticide pollution on the risk of schistosomiasis requires also understanding how pesticides might affect the free-swimming life-stages of the pathogen *Schistosoma* itself.

This study investigated effects of the insecticides imidacloprid and diazinon on larvae of *Schistosoma mansoni* and its interaction with the intermediate host *Biomphalaria pfeifferi*. Both insecticides are common in freshwater bodies in western Kenya (Kandie *et al.*, 2020b). Acute toxicity tests were conducted with miracidia and cercariae to assess the median effective concentration (EC50) at which half of the test organisms are immobilised. Based on these results I assessed whether miracidia host seeking is affected at lethal concentrations (ca. 100 % of the EC50) and at sublethal concentrations (ca. 10 % of the EC50). After successful infection, a single miracidium produces thousands of sporocysts within a snail that are then shed as cercariae. Therefore, maturation and replication of *Schistosoma* relies on nutrient

supply from the snail (Gerard, 1992). As a consequence, sporocysts are indirectly susceptible to environmental conditions such as pesticides that affect the energetic reserves but also the immune system of the snails (Stirewalt, 1954). Additionally, sporocysts may be directly affected by pesticide residues when they enter the body of snails. As such, I assessed whether the maturation of e to cercariae within the host snail *Biomphalaria pfeifferi* is disrupted when the host snail is exposed to pesticide pollution after being parasitized.

4.2 Materials and Methods

4.2.1 Study location

All experiments were conducted at the International Centre for Insect Physiology and Ecology (*icipe*) Thomas Odhiambo Campus (TOC), Mbita, Kenya.

4.2.2 Snail collection and rearing

Biomphalaria snails were collected from the shores of Lake Victoria with a snail catcher and a pool net. Species identification was done with a field identification key (Kristensen, 1987). Collected snails were placed in open plastic containers along with some vegetation from the collection site for shade and cooling during transport. Water was avoided during transport to avoid excess mortality due to warming. In the laboratory, snails were placed in large plastic tubs (45 x 35 x 28 cm) with 5 litres of lake water and reared with boiled kale (*Brassica oleracea L*) and tropical fish food. These tubs were kept at ambient conditions in a greenhouse with netting screened walls at *icipe* TOC. The day after collection, snails were placed individually in 24 well plates and exposed to indirect sunlight for two hours to cause shedding of cercariae, similar to what was done by Opisa *et al.* (2011). After two hours, the well plates were observed under a dissecting microscope (Zeiss AxioCam5 100–400x) for *Schistosoma* cercariae which would indicate which snails were infected. Infected snails were separated to be used to produce the cercariae needed for experiments; but reared similarly to uninfected snails in aerated, dechlorinated water and fed with boiled kales. Uninfected snails were reared for an additional 5 weeks before rechecking for cercarial shedding, after which uninfected snails were considered fit for experiments requiring infection such as the miracidia host seeking and sporocyst development assays.

4.2.3 *Schistosoma* cercariae collection

Cercariae were obtained from *Schistosoma* positive snails. On the days of experiments, the snails were placed in 24 well plates under artificial light at 9 am to allow for cercariae

shedding for two hours before setting up assays. The well plates were then observed under a microscope (Zeiss AxioCam5 100–400x), the snail was removed, and cercariae were pipetted into the test containers for experiments as described in the acute toxicity tests section below. After the experiment, the snails used to shed the cercariae were placed in a freezer to kill them.

4.2.4 *Schistosoma* miracidia collection

Miracidia were obtained from *Schistosoma* eggs extracted from stool samples from primary school children with due consent from both parent and child, and ethical approval from the relevant national authorizing body. Over the course of the experiment, 145 children were recruited for screening from Kombe and Wasulwa A villages in Homa Bay County and Katito, Kisumu County, respectively. Stool samples of about the size of a pea were obtained from the children and tested for infection through the Kato-Katz method (Katz *et al.*, 1972; WHO, 2012). Briefly, the stool was placed on a template that approximates the sample to about 43 mg per slide. The samples were then pressed with a cellophane strip coated with Malachite green dye, and slides were observed under a compound microscope (Axiocam ERc5s at 400x magnification) to look for *Schistosoma mansoni* eggs. The number of eggs per gram (epg) for each slide was also counted. Forty-five children were found to be positive, of which the highly positive (ones with higher egg counts, those with 200 epg or more) were recruited to provide additional stool samples to supply eggs as a source of eggs for miracidia while those with low egg burdens were immediately treated with praziquantel, according to Ministry of Health guidelines using a Ugandan-model dose pole (Sousa-Figueiredo *et al.,* 2012). The stool was collected in plastic containers with lids sealed with cling film. Upon receiving, the stool samples were flushed through sieves with gradually smaller sizes (212, 180, 150, 45µms) using 8.5% saline solution to harvest eggs and ensure they do not hatch. The sieved eggs were stored overnight in falcon tubes with saline, and experiments were conducted the day after egg collection to ensure their viability was not affected by storage time or overexposure to cold temperatures. When miracidia were needed for experiments, the falcon tubes with the eggs were poured into 5 litres of bottled water in a large conical flask. The flask was then covered with a piece of aluminium foil and left to sit on the bench near a window for 2 hours to allow the ova to hatch into miracidia, and the phototropic miracidia to move up the water column to the water surface, where they were then collected using a pipette and transferred into a petri dish and utilised in the experiment. The children who provided samples were treated with a single dose of praziquantel (40mg/kg) under the supervision of a qualified and competent clinician.

4.2.5 Ethical considerations

I required the use of *Schistosoma mansoni* and *Schistosoma haematobium* eggs*,* which are acquired from sieving urine and faecal samples from infected patients. This is routinely done during surveys of infected schoolchildren in the area and with due consent and ethical approval (appendices E1-E4), eggs were collected during the survey and any children found to harbour the parasite were given a dose of Praziquantel by a medical professional at hand.

4.2.6 COVID-19 considerations

In December of 2019, a new strain of coronavirus started infecting humans in Wuhan, China. The disease spread rapidly across the globe, leading to the World Health Organization declaring the disease, henceforth named Corona Virus Disease 2019 (COVID-19). As such, many activities had to be reconsidered, adapted, or excluded due to feasibility challenges arising from the pandemic. As the country was brought to lockdown – schools were shut and citizens were advised to stay at home and minimise contact, with a curfew also enforced, and travel within counties restricted based on transmission rates at various times. These impeded our collection of *Schistosoma* eggs from human patients and snails from certain sites across county borders, which resulted in the exclusion of Bulinus snails and *Schistosoma haematobium* during the experiments in chapter 4, as the Mbita area lake shore consisted of *Biomphalaria* host snails almost exclusively. This also resulted in an observable lack of *Schistosoma haematobium* infections for experiments to be conducted on.

Collection of *Schistosoma* eggs from schoolchildren also required special care, as to minimise contact during this difficult period, where the airborne virus can be transmitted by close contact or through airborne droplets. Community chiefs were recruited to assist in stool sample collection, as they were often in contact with the residents to deal with day-to-day issues arising and spoke the local language. Therefore, I armed them with personal protective equipment and sanitizer and a protocol to minimise contact with patients. The chiefs recruited children by first approaching parents of their respective villages regarding the research ongoing, the possibility of joining the research, and the benefits which were free testing for schistosomiasis for willing participants and free deworming if positive. Parents who consented to their children participating were then approached by chiefs to explain to the child their roles in the research – providing faecal samples for testing, and if positive, larger samples for egg harvesting. Once the children had also understood the research and consented, parents were provided with stool sample collection vials and PPE. Samples collected were picked up by the chiefs and brought to icipe's laboratory in Mbita where the stool was analysed for *Schistosoma* infection by Kato-Katz and discarded in well labelled

trash bags as hazardous material, which was then incinerated. Positive children were contacted by telephone and asked to visit the chiefs' offices where they were separated six feet apart before being individually treated. Unnecessary contact was thus avoided at every juncture.

4.2.7 Insecticides

I tested the effects of two insecticides with different modes of action, the neonicotinoid imidacloprid and the organophosphate diazinon. Both compounds are among those that typically drive the overall risk of agricultural pesticides to freshwater invertebrates in the study area (Kandie *et al.*, 2020b). Imidacloprid was provided with the formulated plant protection product Loyalty® 700 WDG (distributed by Greenlife Crop Protection Africa, Nairobi; manufactured by Shandong United Pesticide Industry China) containing 700g imidacloprid / kg as the active ingredient. Diazinon was provided with the product Diazol® 60 EC (emulsified concentration, repacked and distributed by Laibuta Chemicals Ltd, Nairobi, an insecticide of the chloronicotinyl class; and containing an insecticide that contains diazinon 600g / kg, an organophosphate, as an active ingredient. Both formulated products are commonly sold in the study area. Stock solutions based on the required active ingredient concentration were prepared the night before the experiments. The stock solutions were then left to stir overnight in amber glass bottles covered with foil and used to produce the remaining concentrations through dilutions the morning of experimentation. Fresh stock solutions were prepared the day before every experiment.

4.2.8 Miracidia and cercariae acute toxicity tests

Miracidia and cercariae are the free-swimming, host-seeking life stages of *S. mansoni*. They are in direct contact and thus vulnerable to pesticides in freshwater bodies, and therefore of primary interest. Due to similarity in function, miracidia and cercariae experiments were conducted in a similar manner. Acute toxicity tests were conducted in a temperature-controlled room at 18 ºC where 10 individuals of either the miracidia or cercariae were placed together in petri dishes with 2 ml of the test compound (i.e., imidacloprid or diazinon). Survival of the organisms was recorded after 6 and 12 hours under a dissecting microscope (Zeiss AxioCam5 100-400x).

Ten miracidia were placed in petri dishes with increasing concentrations of each compound (control, 1, 4, 14, 55, 209 µg/L) and the experiment was replicated thrice. Similarly, 10 cercariae were placed in increasing concentrations of each compound (control, 4, 14, 55, 209, 792 µg/L) and replicated thrice. Test concentrations were based on preliminary experiments

such that they covered the range of 5-95% mortality to estimate the median effective concentration required to immobilize 50% of the individuals (EC50) in 12 hours.

Both acute toxicity tests contained organisms from a number of hosts and mixed together, such that cercariae were collected from different snails and miracidia were collected from egg batches from different children to account for the variability between hosts. Experiments were conducted in triplicate at ambient room temperature. Due to high mortality in the controls, experiments were also repeated in a temperature-controlled room at 18°C.

4.2.9 Miracidia host-seeking assay

Miracidia hatch from the eggs and need to find a suitable snail host within 24 hours (Wilson & Carter, 1982). During this period, the miracidia have limited energetic reserves. They can navigate in water to find their host (Chernin, 1970; Wilson & Carter, 1982). To investigate whether pesticides can affect this chemotaxis, I carried out an assay that subjected miracidia to different concentrations of pesticides for either two, four, or six hours before allowing them access to a host. Using a pipette under a dissecting microscope, I distributed 540 miracidia to six petri dishes (60 x 20mm, PYREX 1480102D). Two petri dishes served as control, each of the other dishes contained one of the following nominal test concentrations: 4.88 µg/L imidacloprid (~2% of average 6h EC50 for miracidia), 48.8 µg/L imidacloprid (~20% of average 6h EC50 for miracidia h EC50), 1.05 µg/L diazinon (~2% of average 6h EC50 for miracidia), and 10.5 µg/L diazinon (100% of average 6h EC50 for miracidia h EC50). After pesticide exposure for two, four and six hours, respectively, 60 miracidia were collected from each petri dish and distributed into 12 100 ml oviposition cup, (Fig. 4.1). Each cup contained 70 ml bottled water and a single *Biomphalaria pfeifferi* snail, such that each snail was exposed to 5 miracidia. The miracidia were given 6 hours to infect their host snail. After six hours of access to the snail for the miracidia, the snails were removed from the oviposition cup, washed with bottled water and reared in round plastic tubs (48cm diameter) with 2.5L of lake water, in the screenhouse as above, for as three days. The snails were allowed to live the three days to ensure successful infection has taken hold within the snail. After the three days, the snails were frozen to kill. Once dead, the snails were collected and frozen to store the DNA for molecular analysis to confirm successful penetration by the miracidia as described below.

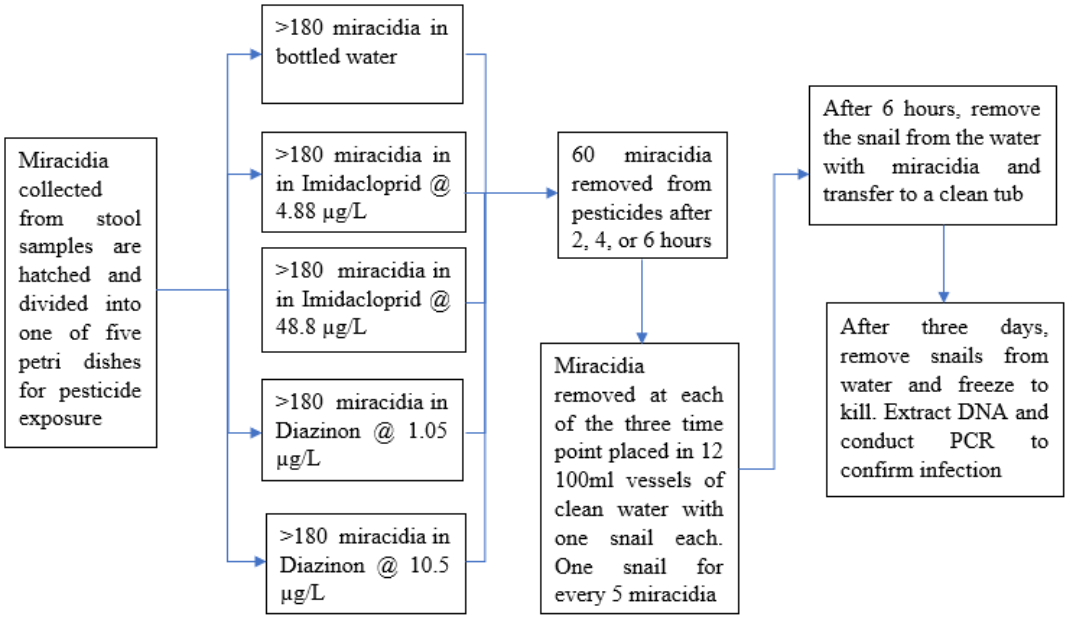
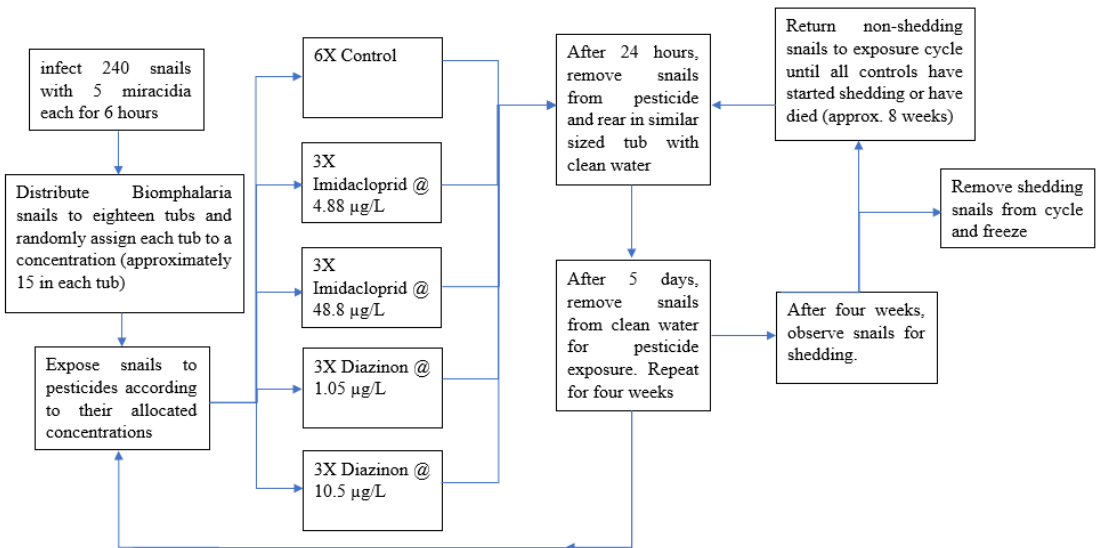


Figure 4.1: Flowchart for the miracidia host seeking assay.

4.2.10 Sporocyst Development Assay

To test the effect of snails being exposed to pesticides on the growth and maturation of sporocysts I first exposed 240 of our youngest non-infected snails with fresh miracidia each in order to infect them.. This was done by placing each snail with 5 miracidia in an oviposition cup with 100 ml of bottled water for 6 hours. I used bottled water here to provide optimum conditions for the miracidia to penetrate and infect the snails. After this infection period, the snails were distributed to six 48cm circular plastic tubs containing 40 snails each. The tubs were then randomly assigned a pesticide concentration they were to receive, either: a control for imidacloprid, imidacloprid at ~2% the average 6h EC50 for miracidia (4.88 µg/L) and 20% theveragee 6h EC50 for miracidia (4.88 µg/L), a control for diazinon, diazinon at ~2% EC50 (1.05 µg/L) and at 20% EC50 (10.5 µg/L). To mimic pulse exposure



in the field, the infected snails were to be exposed to pesticide once a week (Fig 4.2). They were otherwise reared in lake water, as above, that was changed weekly to clean the water and fed boiled kale. Two of the tubs served as controls, the remaining tubs were randomly assigned to one of the following pesticide concentrations: 4.88 µg/L imidacloprid, 48.8 µg/L imidacloprid, 1.05 µg/L diazinon, and 10.5 µg/L diazinon. All test concentrations were below 0.01% of the 24h acute median lethal concentration (LC50) for *B. pfeifferi* (Becker *et al.* 2020). Test concentrations resembled 2% and 20% of the average 6h EC50 of imidacloprid and diazinon for miracidia. Once a week, beginning three days post-infection, the snails were removed from their tubs of lake water and exposed to the described pesticide concentrations for 24 hours in glass bowls (48 cm diameter). After 24 hours, the snails were washed with lake water before being placed back in their original tubs with lake water. Beginning four weeks after repeated exposure, the snails were checked every two days for cercariae shedding by exposing them to artificial light and observing them under a compound microscope. All positive snails were immediately removed for storage in 70% ethanol. Dead snails were also collected in 70% ethanol. Molecular analysis was done on all stored samples to search for *Schistosoma* DNA to confirm the infection status as well as to search for prepatent infections that did not lead to cercarial shedding. The experiment concluded when all snails had died or been collected.

Figure 4.2: Flowchart of the sporocyst development assay

4.2.11 Molecular analysis

To ensure penetration had occurred, snails were tested for *Schistosoma* infection with the help of polymerase chain reaction (PCR) assays which would amplify any *Schistosoma* DNA within the snail. PCR was performed in a total volume of 50 µl containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton® X-100, 200 µM dNTP (Promega, Madison, WI, USA), 2.5mM $MgCl_2$, 0.2 µM of each primer, 1 unit of *Taq* polymerase (Promega, Madison, WI, USA), and approximately 75 ng of schistosome genomic DNA. The thermal cycling profile included an initial denaturation step at 94 ºC for 5 min, followed by 35 cycles of 45 s at 94 ºC, 45 s at 58 ºC, 30 s at 72 ºC and a final step of 7 min at 72 ºC using a GeneAmp 2400 (Applied Biosystems, Foster City, CA, USA) thermal cycler. At point of testing, snails were removed from the freezer and the soft body extracted from the shell using forceps, and cut into small pieces. The bodies were then transferred to an Eppendorf tube and homogenised using a motorised homogeniser, and DNA was extracted using standard protocols (Amarir *et*

al., 2014). The DNA obtained was then amplified using conventional PCR (Sady *et al.*, 2015) using the following primers: ShbmF (5′-TTTTTTGGTCATCCTGAGGTGTAT-3′), ShR (5′-TGATAATCAATGACCCTGCAATAA-3′) and SmR 5′-TGCAGATAAAGCCACCCCTGTG-3′). Amplicons were separated in a 10% agarose gel and visualised in a UV chamber (ingenius syngene bio imaging) and indicated presenceafter staining with ethidium bromide and 100bp DNA ladder used to determine the product sizes. Presence of *Schistosoma* DNA would be confirmed successful by a band on the agarose gel at 250 bps, which would confirm miracidia penetration of a snail (Fig 4.S1)

4.2.12 Data analysis

Estimates of the effective concentration-response immobilization to 50% (EC50) of miracidia and cercariae were determined using the drc package. Prior to estimating the EC50 of the pesticides on cercariae, the effect of temperature and its interaction with concentration and time was assessed using glm. Sporocyst dataset was analysed with generalized linear mixed effect model: with tubs as random variable and concentration as fixed factor. Data on percentage of infected snails was analysed using generalized linear model with logistic distribution, where exposure and concentration were used as fixed variable and infection as response variable. Separation of means were performed for factors that showed significant differences using the lsmeans package (Length, 2015) on the Tukey adjusted p–values. Datasets were analysed using R statistical software (R Core Team 2020).

4.2.13 Ethical Clearance

Ethical Clearance was granted from the Kenya Medical Research Institute's (KEMRI) Scientific and Ethical Review Unit (SERU) to collect stool samples from schoolchildren to obtain miracidia for experiments (KEMRI/SERU/CBRD/194/3836).

4.3 Results

4.3.1 Miracidia and cercariae acute toxicity tests

The life span of miracidia and cercariae is limited to approximately 24 hours, such that I observed a drastic reduction in survival of miracidia after 12 hours. Thus I limited focused the analyses of EC50s to effects after exposure for 6 and 12 hours rather than for 24 hours which is more common in ecotoxicological testing of other species. I found the average exposure to imidacloprid immobilized 50% of miracidia at 15.47±1.74 µg/L after 12 hours (Fig 4.3A) and at 116.15±11.45 µg/L after 6 hours (Table 5.1; Fig 4.4A). Exposure to diazinon immobilized 50% of miracidia at an average of 20.91±1.19 µg/L after 12 hours (Fig 4.3B) and at 54.84±0.54 µg/L after 6 hours (Table 4.1; Fig 4.4B). Cercariae showed greater tolerance than miracidia to imidacloprid, with an average EC50 to imidacloprid of 337.12±67.21 µg/L at 12 hours (Fig 4.5A) and 398.40±24.57 µg/L at 6 hours (Fig 4.6A). Tolerance for diazinon was lower for cercariae for the first 6 hours, with an EC50 to diazinon of 24.79±2.26 µg/L (Table 1; Fig 4.5B) and reduces to a similar EC50 to that of miracidia at 12 hours at 14.82±0.39 µg/L (Fig 4.6B; Table 1). The first experiments were conducted at 25°C where I observed high mortality in the controls, thus subsequent experiments were done in a temperature-controlled room. In the final calculations only replicates where >70% of controls survived were considered, except for when two out of three replicates had high mortality in the controls, in which case all three replicates were discarded. Temperature had no significant effect (stats) nor interaction of temperature, concentration and time (stats) (χ^2=0.361 df=1 p=0.54794).

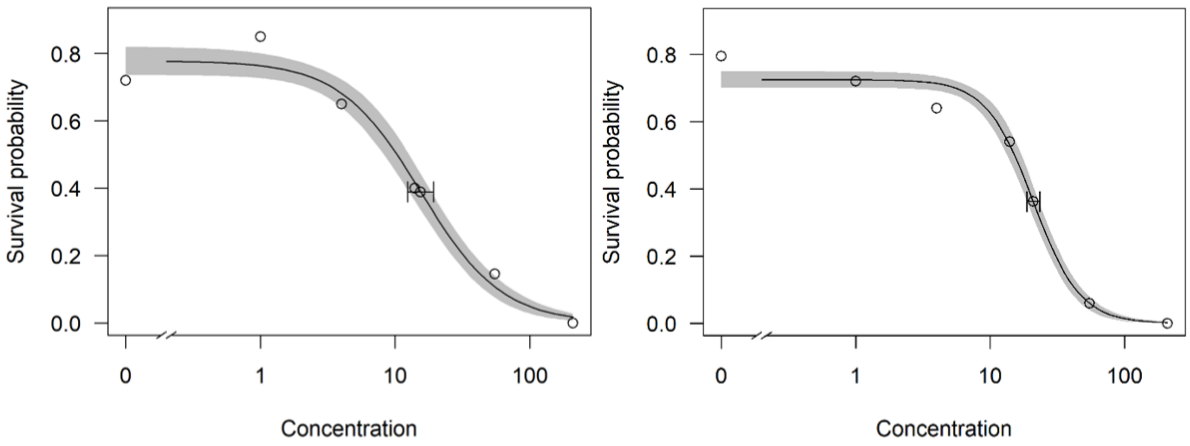


Figure 4.3 A and B: Dose-response curves for *S. mansoni* miracidia after exposure of 12 hours to (A) imidacloprid and (B) diazinon. Data points represent average survival, solid lines show fitted observed concentration−response relationships, and the shaded areas correspond to the 95% confidence intervals.

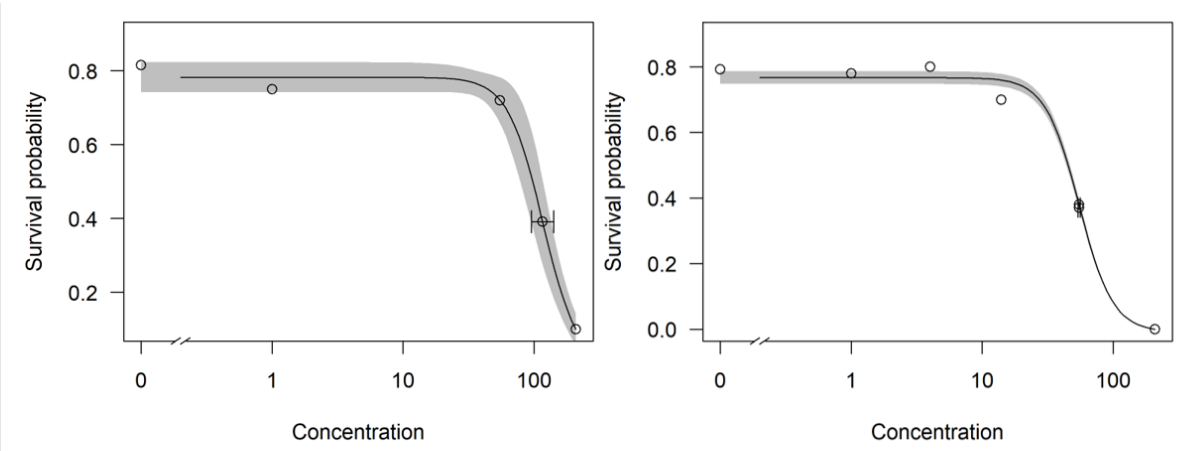


Figure 4.4 A and B: Dose-response curves for *S. mansoni* cercariae after exposure of 6 hours to (A) imidacloprid and (B) diazinon. Data points represent average survival, solid lines show fitted observed concentration−response relationships, and the shaded areas correspond to the 95% confidence intervals.

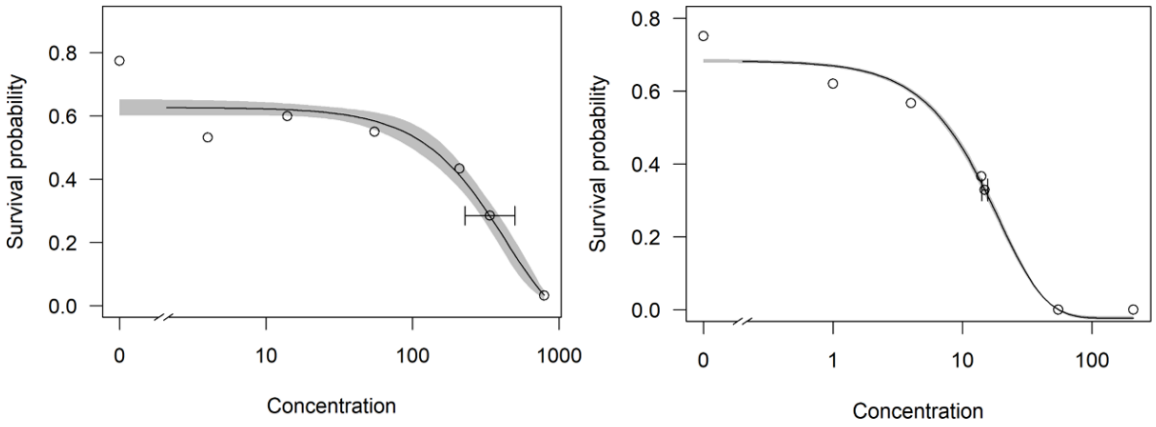


Figure 4.5 A and B: Dose-response curves for *S. mansoni* cercariae after exposure of 12 hours to (A) imidacloprid and (B) diazinon. Data points represent average survival, solid lines show fitted observed concentration−response relationships, and the shaded areas correspond to the 95% confidence intervals.

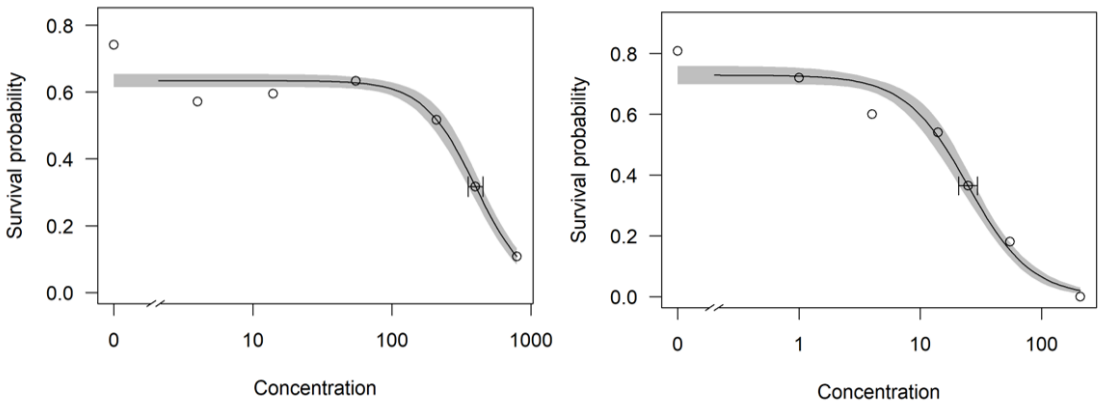


Figure 4.6 A and B: Dose-response curves for *S. mansoni* cercariae after exposure of 6 hours to (A) imidacloprid and (B) diazinon. Data points represent average survival, solid lines show fitted observed concentration−response relationships, and the shaded areas correspond to the 95% confidence intervals.

Table 4.1: Average EC50s of miracidia and cercariae across for imidacloprid and diazinon at 6 and 12 hours.

Life stage	Pesticide	6hrs	12hrs
		EC50 (± SE)	EC50 (± SE)
Miracidia	Imidacloprid	116.15±11.45	15.47±1.74
	Diazinon	54.84±0.54	20.91±1.19
Cercariae	Imidacloprid	398.40±24.57	337.12±67.21
	Diazinon	24.79±2.26	14.82±0.39

4.3.2 Miracidia host-seeking assay

The effect of pesticide exposure on the infectivity of miracidia was identified based on the number of snails that were PCR positive for DNA. Miracidia were placed in bottled water (control), low (2% EC50 of imidacloprid or diazinon) or high doses (20% the average EC50 of imidacloprid or diazinon) for up two, four or six hours before access to a snail in bottled water. Exposure time did not significantly affect the percentage of infected snails ($\chi^2 = 0.779$, df = 2, p = 0.6773), and there was no significant interaction of exposure time and concentration ($\chi^2 = 10.523$, df = 8, p = 0.2302). However, the concentration significantly affected the percentage of infected snails with treatment imidacloprid ($\chi^2 = 20.255$, df = 2, $p < 0.0001$) and diazinon ($\chi^2 = 8.564$, df = 2, p = 0.0138) as the high concentrations of both

imidacloprid and diazinon reduced the proportion of infected snails (Fig 4.5). While these analysis were based on a few data points (Supplementary table 4.S2), the results show that miracidia are have reduced infectiveness in polluted environments, but the few infections show that transmission is not completely eliminated.

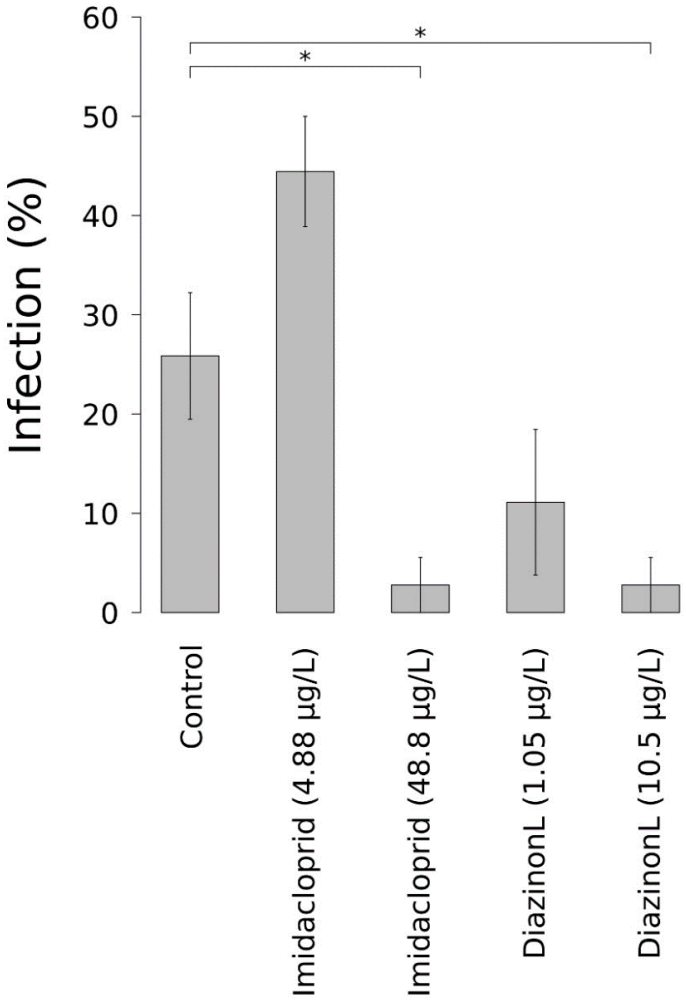


Figure 4.7: Percentage of infected snails when exposed to imidacloprid and diazinon at 10 and 20% the average EC50 for miracidia at 6 hours. Low concentrations did not statistically-significantly reduce infections but high concentrations approximately at their EC50s did.

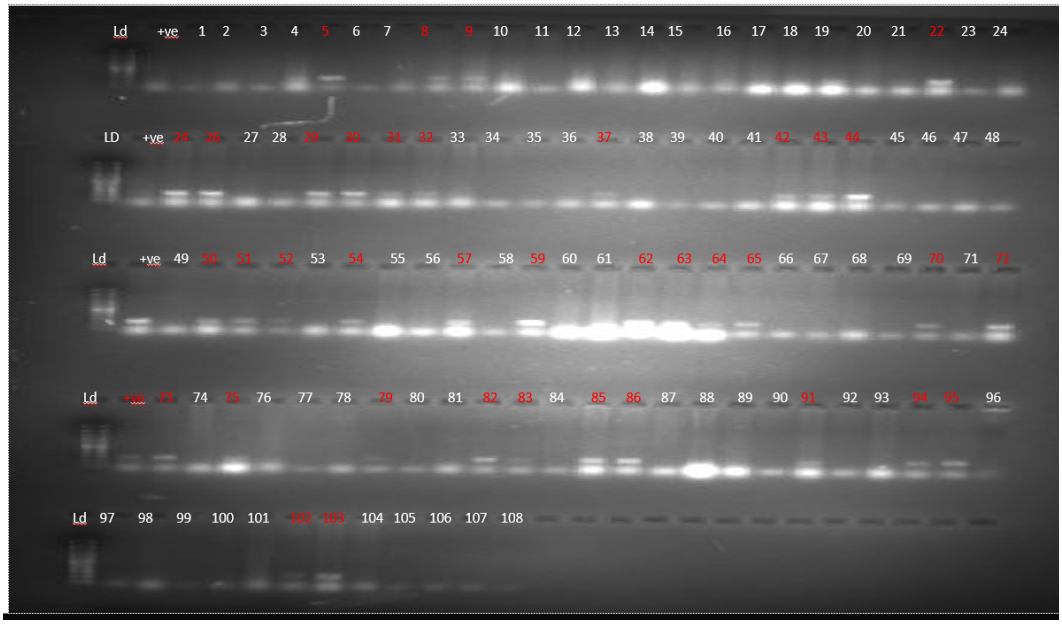


Fig 4.8: Gel image of the PCR results of the miracidia host seeking assay for imidacloprid. The image shows the results for controls (well 1-12= controls in substrate for 2 hours, 13-24= controls in substrate for 4 hours and 24-36= controls in substrate for 6 hours) versus those exposed to imidacloprid at 2% the average EC50 for miracidia at 6 hrs (37-72, arranged for time as controls) and those exposed to imidacloprid at 20% the average EC50 for miracidia at 6 hrs (73-108). Each row starts with a DNA ladder and a positive control (250 bps) except the bottom row which does not have the positive control.

4.3.3 Sporocyst development assay

The effect of weekly pesticide exposure on the development of sporocyst within the snail was identified based on the number of snails shedding cercariae after five weeks. Snails were placed in bottled water (control), low (2% average EC50 of imidacloprid or diazinon) or high doses (20% average EC50 of imidacloprid or diazinon) weekly and no statistically significant effect could be observed in the number of infected snails between controls and those exposed to imidacloprid (χ^2=0.8094, df=2, p=0.3683) or diazinon (χ^2=0.053, df=2, p=0.815 (Fig 4.9). There was also no statistically significant change in the number of infected snails when the dataset was limited the first day of shedding imidacloprid (χ^2=0.069, df=1, p=0.793) or diazinon (χ^2=0.0036, df=1, p=0.952). There was no statistically significant change in the number of snails throughout the course of the experiment (4.10).

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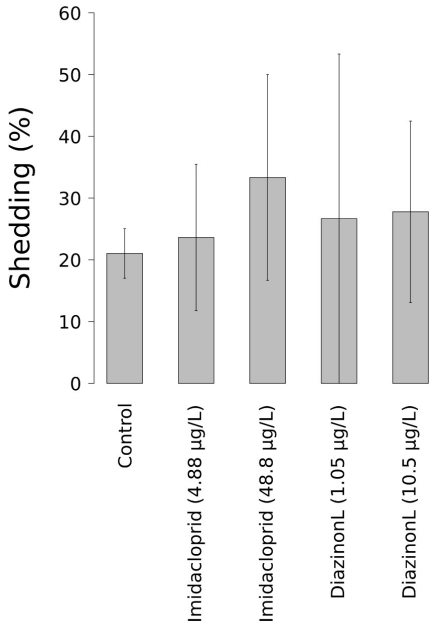


Figure 4.9: Percentage of snails found to be shedding cercariae after 8 weeks of pesticide exposure with imidacloprid and diazinon at 10 and 20% the average EC50 for miracidia at 6 hours. Pesticides had no effect on sporocyst development.

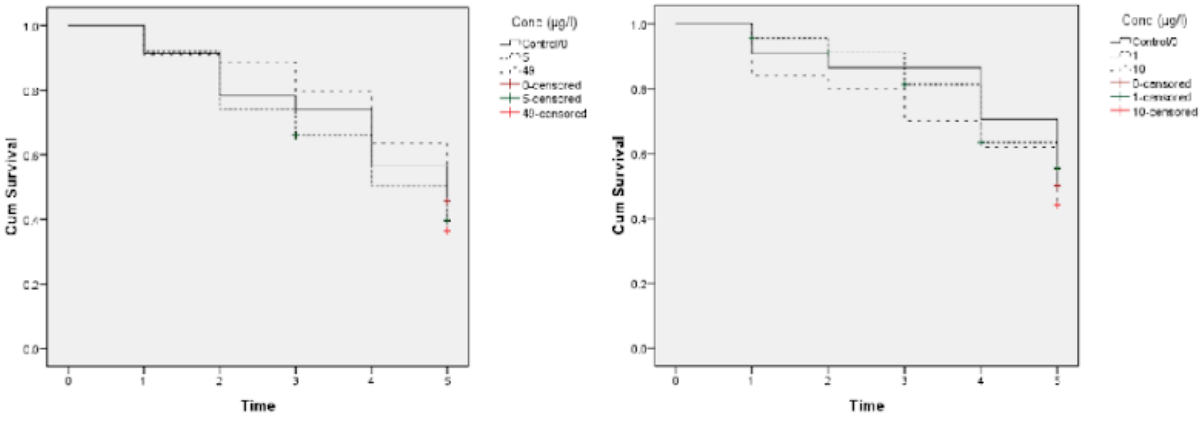


Figure 4.10 The survival of *Biomphalaira pfeifferi* under of five weeks of pulse exposure of Imidacloprid (left) and Diazinon (right) respectively. The snails were placed in the pesticides (except controls, solid lines) for 24 hours after which they were otherwise in bottled water for the rest of the time. Snails had been infected and the survivors at the end were checked for S*chistotoma* cercariae beginning the five-week period up to their deaths. Snail survival was not significantly affected by Imidacloprid at 2% (ChiSq= 0.056, P=0.812) nor 20% ChiSq= 0.717 P=0.397) our observed average EC50 concentrations to *Schistosoma* miracidia and 10 (ChiSq= 0.455, P=0.500) and 20% (ChiSq= 0.118, P=0.731) EC50s.

4.4 Discussion

The experiments above show that the imidacloprid and diazinon concentrations observed in natural water systems may not kill the *Schistosoma* free-swimming life-stages directly, affect their development within the snail, or have an effect on the miracidia's ability to seek out or infect their host snails. Sublethal concentrations were observed to be able to reduce the number of snails infected with miracidia. However these were at concentrations 565-600 times higher than those observed in the aquatic environment (Kandie *et al.*, 2020a). The data on infectivity and sporocyst development suggest there are no relevant effects on the performance of *S. mansoni* at environmentally relevant concentrations. I further estimated the effective concentrations that immobilise 50% at 6 hours (6hr EC50) and observed that the concentration of diazinon needed to effectively inactivate the miracidia was with three orders of magnitude higher than observed field-concentration.

The EC50s observed for the two life-stages were used to determine the concentrations used in the next two experiments, which were to be a sublethal concentration at 20% the EC50 for miracidia at 6 hrs, 565 times the amount of diazinon that could be found in the environment, or 600 the amount of imidacloprid; and a sublethal concentration 10 times lower than the first, at 2% the EC50 for miracidia at 6hrs, roughly 56 times the amount of diazinon that could be found in the environment, and 60% the imidacloprid recorded in the area (Kandie *et al.*, 2020a). The EC50s of the *Schistosoma* for diazinon fall 1000 times lower than that of the host snails *B. pfeifferi*, and one hundred thousand times lower for imidacloprid. However, EC50s of the *Schistosoma* are higher than those of standard test organisms, with concentrations of diazinon needed to immobilize miracidia being 10 times higher than the 48 h EC50 *D. magna*. The EC50s of *Schistosoma* to imidacloprid was similar to the 96 h EC50 *C. riparius* (Lewis, 2016). While the concentrations are not directly comparable as the exposure time are different, thus overestimating the EC50s of *Schistosoma*, the estimates reveal that the miracidia may be able to survive in waters where the host snails' competitors cannot (Becker *et al.*, 2020). Furthermore, the concentration necessary to cause the observed reduction in miracidia would be highly localised, and dilute as it diffuses within the river waters and miracidia would continue to be released even after the peak exposure time has elapsed, therefore, it is likely that many miracidia in the field will not be exposed to concentrations harmful to them. Though it should be noted that ecotoxicological investigations on macroinvertebrates reveal negative responses to pesticides in the field at environmental concentrations, despite their tolerance in the laboratory being much higher. This is due to a number of factors, such as additional stressors (Cornejo *et al.*, 2019) or

mixture toxicities (Weisner *et al.*, 2021), among others. This could also be the case for *Schistosoma*.

The sporocyst development assay showed little difference in the proportion of snails shedding between those exposed to pesticides and the controls. While the lethal concentrations can reduce proportion of snails infected, these concentrations are unable to prevent the maturation and development of miracidia into sporocysts and cercariae within the snail. This suggests that environmentally relevant conditions are unlikely to directly impact the transmission of disease in the field. Considering that pesticides have been shown to indirectly benefit host snails, increasing their abundance and dominance in polluted environments (Becker *et al.*, 2020), the overall effect of pesticides may be to increase the risk and transmission of the parasite and disease in polluted areas. Thus, pesticide mitigation measures should be taken in at-risk areas to prevent further exacerbation of the disease. Though it must be noted, the snails used for this experiment were field collected and thus had to reared for five weeks to ensure they were free of infection before experimenting on them. The death toll during these five weeks severely reduced the number of snails usable for the experiment. The use of miracidia is dependent on ethical approvals and infected children. The presented results hence need to be interpreted with the limitations in mind and will require further validation. The developed bioassays can be used for testing pollutants at different concentrations going forward to elucidate the overall effect pesticides have on the various life-stages.

In conclusion, more research is needed with additional agrochemical compounds to elucidate the true effects that would be observed in the field. The link between temperature and pesticides needs to be further explored, considering the varying results seen in our acute toxicity tests, with imidacloprid being more potent at lower temperatures while diazinon was less potent. The sporocyst development should also not be considered an invulnerable stage from the above experiments, as our pesticide exposure began the week of infection. In the field, the snails may be exposed from the beginning of their lives, and thus there may be an accumulation of the pesticides by the time natural infection by miracidia occurs, which may, in turn, reduce the proportion of shedding snails.

CHAPTER FIVE

MONITORING PESTICIDE POLLUTION IN STREAMS IN KENYA

Abstract

Pesticides are washed from agricultural fields into adjacent streams where even short-term exposure causes long-term ecological damage. Detecting pesticide pollution in streams thus requires the expensive monitoring of peak concentrations during run-off events. Alternatively, exposure and ecological effects can be assessed using the $SPEAR_{pesticides}$ bioindicator that quantifies pesticide-related changes in the macroinvertebrate community composition. $SPEAR_{pesticides}$ has been developed in Central Europe and validated in other parts of Europe, Australia and South America; here I investigated its performance in East African streams. With minimal adaptations of the $SPEAR_{pesticdes}$ index, I successfully characterized pesticide pollution in 13 streams located in Western Kenya. The East African $SPEAR_{pesticides}$ index correlated well with the overall toxicity of 30 pesticides (maximum toxic unit = maximum environmental vs. median lethal concentration) measured in stream water (R^2 = 0.53). Similarly, the $SPEAR_{pesticides}$ index correlated with the risk of surface run-off from agricultural fields (as identified based on ground slope in the catchment area and the width of protective riparian strips, R^2 = 0.45). Unlike other bioindicators designed to indicate general water pollution, $SPEAR_{pesticides}$ was independent of organic pollution and highly specific to pesticides. In 23% of the streams, pesticides exceeded concentrations considered environmentally safe based on European first tiered risk assessment. Increasing contamination was associated with considerable changes in the macroinvertebrate community composition. I conclude that pesticides need to be better regulated also in developing countries. $SPEAR_{pesticides}$ provides a straightforward and cost-efficient tool for the required monitoring of pesticide exposure in small to medium streams.

5.1 Introduction

In 2020, the worldwide application of agricultural pesticides is expected to increase from 2 million tonnes to 3.5 million tonnes annually (Sharma *et al.,* 2019). Pesticide pollution is considered one of the main drivers for the global decline in the abundance and diversity of insects, plants and birds (Beketov *et al.* 2013; Geiger *et al.* 2010; Sánchez-Bayo & Wyckhuys 2019). There is increasing evidence that the pesticide-driven impairment of biocenoses also affects valuable ecosystem services ranging from pollination (Rundlöf *et al.,* 2015) to leaf-litter degradation (Schaefer *et al.,* 2011b) and to the biological control of agricultural

pests (Roubos *et al.,* 2014; Talebi *et al.,* 2008) and of pathogens in freshwater (Becker *et al.,* 2020a). For the United States and the European Union, where pesticides are used on a large scale, extensive literature on exposure and effects in the environment is available from academic research and from regulatory risk assessment (Sharma *et al.,* 2019). In developing countries, regulation of plant protection products is often poor and information on pesticide pollution is scarce, though recent evidence suggests that pesticide usage has been increasing (Sharma *et al.,* 2019). As agricultural producers grow more conscious about the use of synthetic pesticides and their toxic effects (Hernández *et al.*, 2013), more effort in the assessment and mitigation of pesticide pollution is urgently needed.

Freshwater macroinvertebrates in small streams are at particular risk, since streams collect pesticide loads from agricultural fields in the catchment area (Münze *et al.,* 2015). Moreover, many freshwater arthropods are highly sensitive to insecticides and fungicides (Morrissey *et al.,* 2015; Van Dijk *et al.,* 2013; Von Der Ohe & Liess 2004). However, the detection and quantification of pesticides in streams is challenging, as exposure occurs typically in short pulses due to spray drift and particularly due to surface run-off from agricultural fields following heavy rainfall (Liess *et al.,* 1999). Such short-term exposure peaks (in the range of hours) drive long-term effects on the macroinvertebrate community for months (Beketov *et al.,* 2008; Cold & Forbes, 2004; Liess & Schulz, 1999). Therefore, pesticide measurements from grab samples of stream water and sediment at random time points tend to considerably underestimate the magnitude of pesticide exposure. Realistic environmental monitoring in streams must capture the exposure peaks by run-off event-triggered sampling (Liess *et al.,* 1999) or by continuous passive sampling over extended periods of time (Münze *et al.,* 2015). However, such studies are labor-intensive and expensive (Vrana *et al.*, 2005). In addition to the chemical analysis of samples for many compounds, samplers need to be installed, protected, and regularly accessed in remote areas. These challenges limit the feasibility of monitoring pesticide pollution based on chemical analyses, particularly in developing countries.

As an alternative approach, pesticide exposure can be indirectly derived from its observed effects on the macroinvertebrate community composition. The $SPEAR_{pesticides}$ ("SPEcies At Risk") bioindicator has been developed to quantify decreases in the proportion of those taxa considered to be vulnerable to pesticides, as compared to reference conditions (Liess *et al.,* 2008; Liess & von der Ohe, 2005). For this task, stream macroinvertebrates have been classified as vulnerable or non-vulnerable taxa based on ecotoxicological traits (see methods). Because the $SPEAR_{pesticides}$ index describes the proportion of vulnerable taxa (weighted by

individual number), it has no unit and does not facilitate the identification of individual toxic compounds in the environment. However, $SPEAR_{pesticides}$ values can be translated to an estimated toxic pressure for macroinvertebrates in the more informative form of toxic units (see methods). Since the $SPEAR_{pesticides}$ value is driven by long-term effects, it can be derived from a single community sample per site. $SPEAR_{pesticides}$ thus offers a potential cost-effective technique for the monitoring of pesticide pollution in small to medium streams that may be specifically valuable in developing countries.

$SPEAR_{pesticides}$ has been developed for temperate streams in Central Europe. Thus, the studied species composition, pesticide exposure patterns and ecological conditions may differ from those in other continents such as in sub-Saharan Africa. Malherbe *et al.* (2018) found only a non-significant response of $SPEAR_{pesticides}$ values to pesticide pollution in South African streams. In contrast, $SPEAR_{pesticides}$ has been successfully applied and validated to assess pesticide pollution in various streams ranging from Southern to Northern Europe (Liess *et al.,* 2008), and (after minor modification) in Australia (Schaefer *et al.,* 2011a) and Argentina (Hunt *et al.,* 2017). The $SPEAR_{pesticides}$ concept is based on ecotoxicological traits rather than taxonomic relations and thus provides a mechanistic linkage of pesticide stress and the community response; in contrast to classical taxonomy-based bioindicators, trait-based approaches can overcome issues with natural taxonomic variability and may thus be applicable even across different climatic regions (Menezes *et al.,* 2010). For freshwater macroinvertebrates in the Southern hemisphere, information on ecotoxicological traits is very scarce (Malherbe *et al.,* 2018; Schaefer *et al.,* 2011a). However, Wang *et al.* (2014) found that related saltwater invertebrates from temperate and tropical regions differ only slightly in their acute sensitivity to toxicants. Moreover, $SPEAR_{pesticides}$ can be applied at the family taxonomic level (Beketov *et al.,* 2009), and palearctic and afrotropical streams share most of their macroinvertebrate families (Ochieng *et al.,* 2020). Assuming that families of freshwater macroinvertebrates from temperate and tropical streams may generally share their vulnerability to pesticides, $SPEAR_{pesticides}$ may be therefore used also in sub-Saharan Africa.

Here I applied the $SPEAR_{pesticides}$ bioindicator to macroinvertebrate samples from 13 small to medium streams in Western Kenya. The $SPEAR_{pesticides}$ values were compared to the toxic pressure of 30 pesticides in water samples concurrently collected during the rainy season (Kandie *et al.,* 2020a). Additionally, I tested the specificity of the $SPEAR_{pesticides}$ index for pesticide effects and compared its performance to alternative bioindicators for environmental stressors.

5.2 Materials and methods

5.2.1 Study area

The Lake Victoria South Basin in the western part of Kenya (Fig. 5.1) is characterized by a tropical climate with a major rainy season between March and June and a minor rainy season between October and December. The rainy seasons are separated by dry months, particularly in the lowlands close to the shore of Lake Victoria. The area is densely populated, but poorly developed in terms of infrastructure and sanitation. It is dominated by food production, mainly maize, for local markets with small-scale fields that often range close to the unfortified banks of streams. However, large scale commercial farming also exists in the form of irrigated rice fields, sugarcane and particularly tea plantations (Wambugu & Muthamia 2009). During the rainy seasons, streams turn red from silt loads indicating heavy erosion and a high potential for surface run-off from agricultural fields.

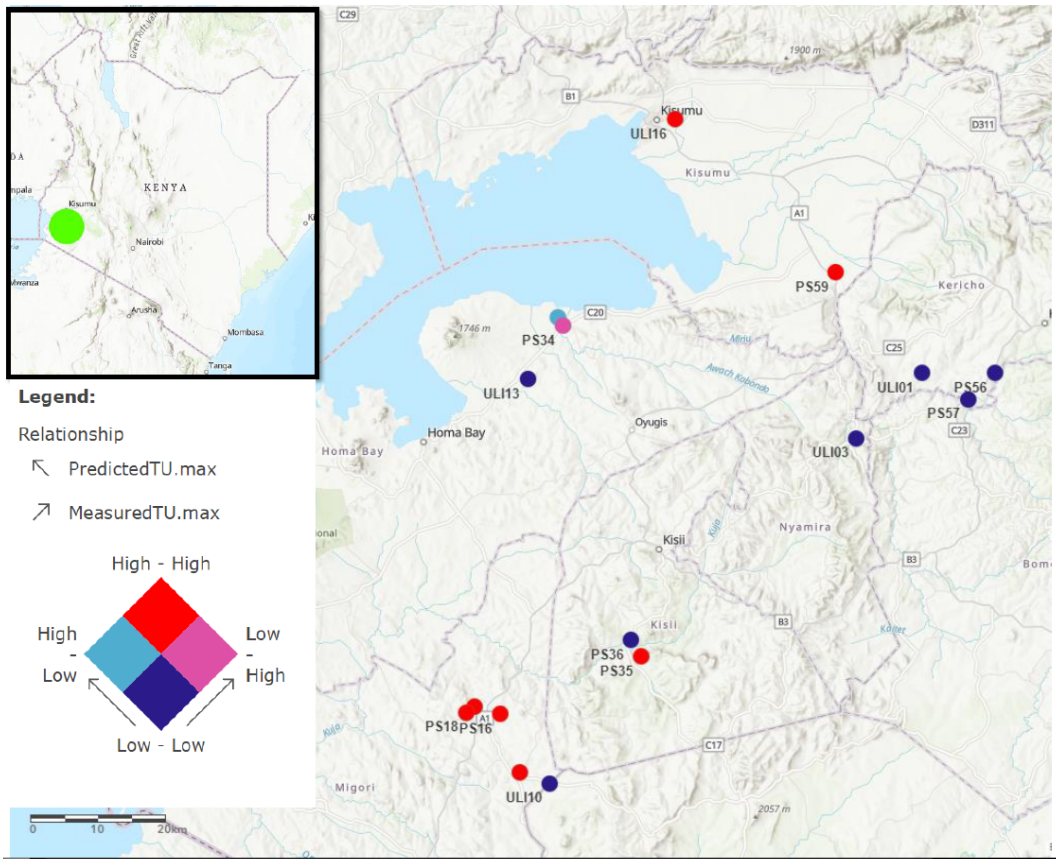


Figure 5.1: Location of the sampling sites in the study area of Western Kenya, East Africa. Site locations are displayed with the site code and colour-filled to annotate the relationship between predicted pesticide pollution (TU_{max}) using $SPEAR_{pesticides}$ and measured pollution in water samples using LC-HRMS in Kandie *et al.* (2020a). Akbar Ganatra.

"$SPEAR_{pesticides}$ study sites". "World Topographical map" and "World Hillshade". February 2nd, 2021. https://arcg.is/1fiDn9.

5.2.2 Pesticide measurements

The sampling and analysis of pesticide residues has been described in detail in Kandie *et al.* (2020a). In brief, 48 sites covering small to large streams, oxbow lakes, irrigation channels, reservoirs and rice fields were sampled during the rainy season in September – October 2017. A single 500 mL water sample per site was collected in a pre-cleaned glass. Solids were allowed to settle for 1 min, before aliquots of 1 mL were transported to the laboratory in amber glass vials at -4°C. Chemical analysis was performed by directly injecting 100 µL of the water sample into a high performance liquid chromatography system (Thermo Ultimate 3000 LC) coupled to a high resolution mass spectrometer (QExactive Plus, Thermo). The water samples were subjected to target screening for 428 chemicals and to suspect screening for 233 additional substances. This analysis included 162 hydrophilic pesticides (active substances and metabolites), but no highly hydrophobic compounds such as pyrethroid insecticides for which different sampling techniques are required. Raw data was processed using Mzmine (Version 2.38, Pluskal *et al.*, 2010) and further confirmation and quantification done using TraceFinder 4.1 (Thermo) as detailed in Kandie *et al.* (2020a). Physicochemical water parameters were measured *in situ* (flow velocity, temperature, dissolved oxygen, phosphates, pH, turbidity) and from additional water samples in the laboratory (nitrate, nitrite, ammonium, carbonate hardness).

In the present study, I only considered pesticides and converted the observed concentrations in the water samples to toxic units in order to quantify the toxic pressure for freshwater macroinvertebrates. The toxic unit (TU; Sprague 1970) describes the environmental concentration *conc* of a pesticide *i* in relation to its median effective or lethal concentration (EC_{50} or LC_{50}, concentration that affects or kills 50 % of individuals in an acute toxicity test)

Eq 1: $$TU_i = \frac{conc_i}{LC50_i}$$

For the TU calculation I generally referred to the LC_{50} for the water flea *Daphnia magna* after constant exposure for 48 h as reported in the Pesticides Properties Data Base (PPDB, 2019). *D. magna* was selected as a reference because it belongs to the more sensitive freshwater macroinvertebrates and because most toxicological data are available for this species due to its use in regulatory risk assessment (Brock & Van Wijngaarden, 2012). However, for some pesticides (particularly neonicotinoid insecticides), *D. magna* turned out to be considerably

more tolerant than other macroinvertebrates (Brock & Van Wijngaarden, 2012). Therefore, we additionally extracted the LC_{50} for the saltwater shrimp *Americamysis bahia* after 96 h exposure from the PPDB data base in case it was available. In cases where *D. magna* was highly insensitive (LC_{50} > 10 mg/L), I additionally took data from the ECOTOX data base (ECOTOX 2019) for the standard test species *Chironomus riparius* and *Hyalella azteca*. If the LC_{50} of *D. magna* was > 10 times higher than that of an additional species observed after 48 h or > 100 times higher after 96 h, I used the most sensitive additional species as reference (mean LC_{50} from different studies in ECOTOX, if available). For metabolites I used the LC_{50} of the parental compound if no toxicity data was available or if the metabolite was less toxic than the parental compound.

The overall toxic pressure from pesticide mixtures in the water samples was summarized as the summed up (TU_{sum})(Sprague 1970) and as the maximum (TU_{max})(Liess & von der Ohe, 2005) toxic unit. TU_{max} and TU_{sum} were set to a minimum threshold of 10^{-4} for sites at which no potentially relevant concentrations were detected; this threshold is in the range of the limit of quantification for the most toxic pesticides in the chemical analysis. Additionally, no effects of pesticides on freshwater macroinvertebrate communities have been generally observed in the field below a threshold range of TU_{max} between 10^{-3} and 10^{-4} (Schaefer *et al.,* 2012).

5.2.3 The $SPEAR_{pesticides}$ concept

The $SPEAR_{pesticides}$ bioindicator has been developed to specifically quantify pesticide-driven deviations in the community structure from those observed under non-polluted reference conditions. Since its first publication in Liess and von der Ohe (2005), $SPEAR_{pesticides}$ has been constantly refined (Beketov *et al.,* 2009; Liess *et al.,* 2008). Here I refer to the latest version 2019.11 that includes some major improvements described in Knillmann *et al.* (2018).

$SPEAR_{pesticides}$ is based on a classification of freshwater macroinvertebrates in" SPEcies At Risk" and in taxa being not at risk based on four traits that describe their vulnerability to pesticides: 1.) The average physiological sensitivity to various pesticides (*s*-value, mean of $\log_{10}$(LC_{50} relative to the LC_{50} of the reference species *D. magna* or *Chironomus sp.*)); 2.) The ability of autochthonous population recovery from reproduction (generation time); 3.) The ability of allochthonous recovery from recolonization (dispersal from non-polluted refuge areas); and 4.) The probability of being actually exposed (e. g. aquatic life stages during the main insecticide application season). Taxa with high sensitivity, low ability of autochthonous and allochthonous recovery and high probability of being exposed have been classified as

being at risk, others as not at risk. A trait database for Central European taxa, linked to a program for the calculation of $SPEAR_{pesticides}$ values is available with the software INDICATE (htpp://www.systemecology.eu/indicate). The database comprises entries for individual species, but also for higher taxa where available data on lower taxonomic levels have been averaged.

The $SPEAR_{pesticides}$ index relates to the proportion of taxa at risk within the macroinvertebrate community and decreases with increasing toxic pressure (Knillmann *et al.,* 2018):

Eq 2: $$SPEAR_{pesticides} = \frac{\sum_{i=1}^{n}\left(4x_i+1\right)\cdot y}{\sum_{i=1}^{n} log_{10}(4x_i+1)}$$

with x_i being the observed number of individuals of taxon *i*, and *y* being 1 for those taxa classified at risk and 0 for taxa not at risk. In this formula, $SPEAR_{pesticides}$ values can range between 0 (no species at risk, indicating high pesticide effects) and 1 (only species at risk). Abundant taxa are down weighted to limit the influence of populations with mass development so that the $SPEAR_{pesticides}$ index increases rather equally with both the incidence and the population density of vulnerable taxa. In order to facilitate interpretation, since version 2019.11, "raw" $SPEAR_{pesticides}$ values obtained from the equation above are divided by the average $SPEAR_{pesticides}$ value observed in non-polluted reference sites (at measured $TU_{max} < 10^{-4}$) from Germany (Knillmann *et al.,* 2018):

Eq 3: $$scaled\ SPEAR_{pesticides} = \frac{raw\ SPEAR_{pesticides}}{reference\ SPEAR_{pesticides}}$$

With $reference\ SPEAR_{pesticides}$ = 0.27. This scaled $SPEAR_{pesticides}$ index ranges from 0 (no species at risk) to 3.7 (only species at risk) and indicates no toxic pressure at values > 1. For European streams, an empirical relationship between $SPEAR_{pesticides}$ and the measured log_{10}-transformed TU_{max} during run-off events has been established to convert $SPEAR_{pesticides}$ values to the estimated toxic pressure (Knillmann *et al.,* 2018; Liess *et al.,* 2008). Because the community composition of Kenyan freshwater macroinvertebrates may differ from those in Germany, both the classification of individual taxa and the conversion to TU_{max} were subjected to revision in the present study (see results).

5.2.4 Application of $SPEAR_{pesticides}$ in the study area

I applied $SPEAR_{pesticides}$ to macroinvertebrate samples collected together with the water samples from Kandie *et al.* (2020a, see above). The macroinvertebrate sampling has been described in detail in Becker *et al.* (2020a). In brief, macroinvertebrates were collected at the

same site and day as the water samples, following a standardised approach adapted from the South African Scoring System 5 (SASS5)(Dickens & Graham 2002). At each stream site, a 50 m stretch was sampled in four quadrants by two persons in parallel. Each quadrant was sampled for 7 minutes using sweep nets for the water surface and littoral habitats, and kick sampling for the benthic macroinvertebrates. Additionally, gravel, soil and mud (GSM) habitats were sampled for 1 minute per quadrant. Organisms were preserved in 70 % ethanol and identified to the family level under a dissecting microscope in the laboratory. $SPEAR_{pesticides}$ v. 2019.11 and the associated estimated TU_{max} were calculated using the software INDICATE v. 1.2.0 (htpp://www.systemecology.eu/indicate).

5.2.5 Sampling site selection

The domain of applicability of the $SPEAR_{pesticides}$ bioindicator is limited to small and medium (semi-)natural streams. $SPEAR_{pesticides}$ is not considered to work in large rivers or temporary streams, in streams with highly degraded morphology or (almost) standing water, and in heavily polluted streams such as waste water treatment plant or mining effluents. Under those conditions, the macroinvertebrate community is expected to differ substantially from those observed in small reference streams, so that changes in the community composition may not be attributed to pesticide pollution.

The East African $SPEAR_{pesticides}$ index has been calibrated using data from 13 streams in Western Kenya. This area is characterized by a tropical climate with two rainy seasons separated by dry months. Based on the widespread applicability of the European $SPEAR_{pesticides}$ index across Europe (Liess *et al.* 2008), I expect that the East African $SPEAR_{pesticides}$ index may work also in various areas across the afrotropical region. However, applications should be verified with chemical analyses on a subset of sites, particularly when climatic conditions differ from those in Western Kenya.

5.2.6 Macroinvertebrate sampling

Macroinvertebrate samples should be collected in seasons when pesticide exposure in streams is expected to be high. In seasons with low exposure, macroinvertebrate communities may partly recover due to recolonization and reproduction which may blur the observable effects of pesticides. E. g., in Central Europe, $SPEAR_{pesticides}$ performs best with samples collected in spring and early summer when most insecticides (the group of pesticides with the highest potential risk) are applied and washed into streams following heavy rainfall. In Western Kenya, I expect that insecticides may be applied rather all year long and collected samples during the two rainy seasons when run-off potential is high. There is no experience yet

regarding how seasonal changes in African macroinvertebrate communities may affect the $SPEAR_{pesticides}$ index. Ideally, a number of samples may be collected from the same sites in different seasons to assess the optimal timing and consistency of effects. However, macroinvertebrates should not be collected during a flooding event when organisms hide or get washed away.

The sampling of macroinvertebrates should follow a standardized methodology to ensure a consistent sampling effort across sites so that samples will be comparable (Figure 5.2). Various microhabitats such as sand, stones, organic matter and aquatic plants should be sampled according to their proportion at a site. International sampling protocols are available e. g. in ISO 10870:2012 (https://www.iso.org/oristandard/46251.html) recommended for monitoring under the EU Water Framework Directive (European Commission 2003), or in the guidelines for the South African Scoring System 5 (SASS5, Dickens and Graham 2002). I used a modified SASS5 sampling protocol described in Becker *et al.* (2020) and in the methods section of the present publication.

Figure 5.2 Macroinverbrate sampling being undertaken with a snail catcher (left) and macroinvertebrate identification using a white tray, forceps and ethanol in collection tubes (right).

5.2.6 Taxonomic identification

Taxa should be generally identified to the family level, either in the field or in the laboratory. The ecotoxicological trait data base for the European $SPEAR_{pesticides}$ index offers optionally higher resolution for some taxonomic groups, based on an operational taxa list from www.freshwaterecology.info. However, no such taxonomic detail is available for African taxa, whereas I found that most of the families in Western Kenya were available in the European data base. Beketov *et al.* (2009) showed that the explanatory power of the $SPEAR_{pesticides}$ index is not significantly lower when calculated from family-level data as compared to species-level data.

The availability of taxonomic literature on East African freshwater macroinvertebrates is limited. I used identification keys provided in Brown (2002), Day *et al.* (1999), Day *et al.* (2001), Day (2001), Day (2002a), Day *et al.* (2002b), Day (2002c), de Moor (2003a), de Moor (2003b), and Harrison (2009). Calculations of the $SPEAR_{pesticides}$ index require that the abundance of each taxon is ratio scaled (absolute individual counts or population density in individuals per m²), i. e. the use of frequency indices should be avoided. Samples with very few taxa (< 5) or individuals (< 30) indicate either issues with the sampling method or a heavily degraded macroinvertebrate community, and may be excluded from $SPEAR_{pesticides}$ calculations.

5.2.7 Calculation of the SPEAR index

I recommend to use the software INDICATE freely available for download at http://www.systemecology.eu/indicate/. Here I refer to the current software version 1.2.0. A manual and background information can be found on the web page. In brief, open the $SPEAR_{pesticides}$ program and enter the monitoring data including name, taxa and abundances for each sample (Figure 5.3).

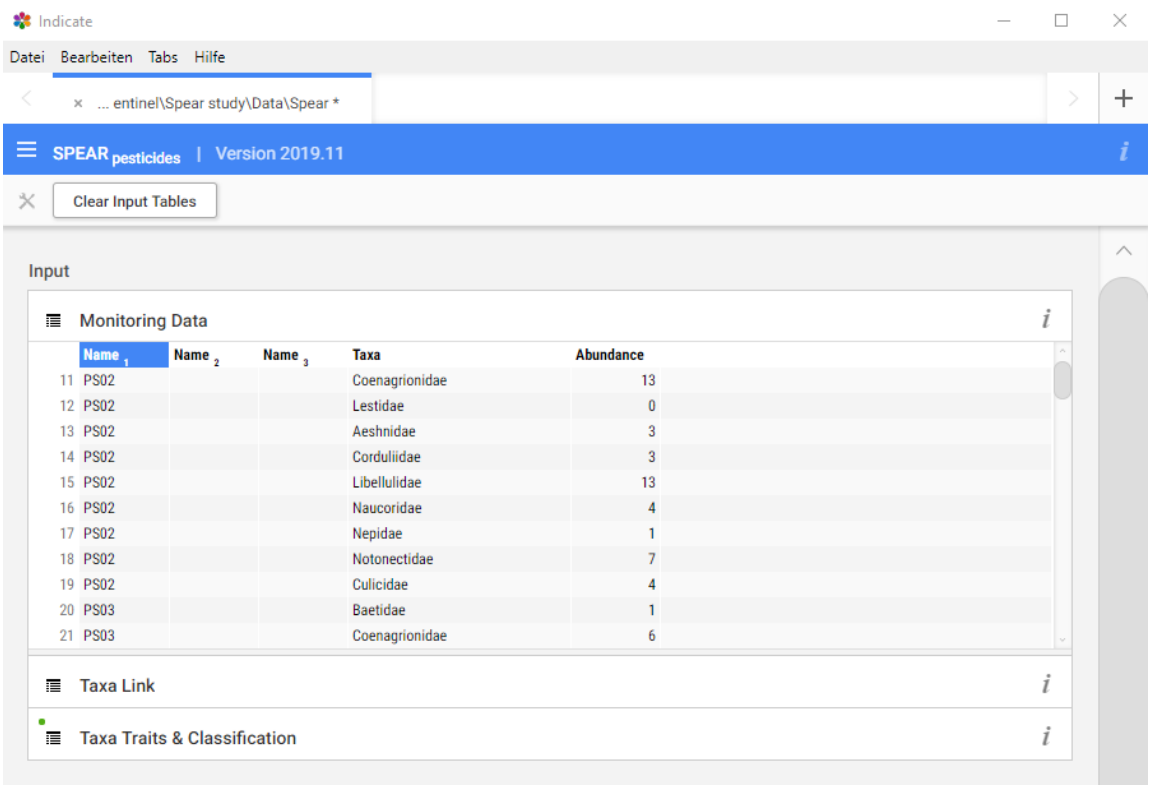


Figure 5.3 The INDICATE program showing the input tables of the macroinvertebrate sampling data. Inputs should include the site name, taxa collected and abundance data of each taxa at each site.

Next, open the button “Taxa Link” and check if the automatic identification of your taxon names by the software was correct. Otherwise, taxon names in the monitoring data can be manually linked to taxa in the ecotoxicological trait data base for $SPEAR_{pesticides}$ calculation. Click on the respective field and enter a new name or choose from the opening dropdown list. Taxa not found in the trait data base should be linked to higher-level taxa, and if not possible, be excluded from $SPEAR_{pesticides}$ calculations (blank field). Note: Some taxa such as “Diptera” are detected by INDICATE but not considered for $SPEAR_{pesticides}$ calculation. Such taxa could not be classified as being “at risk” or “not at risk” due to high trait variation among its species. A warning is given out.

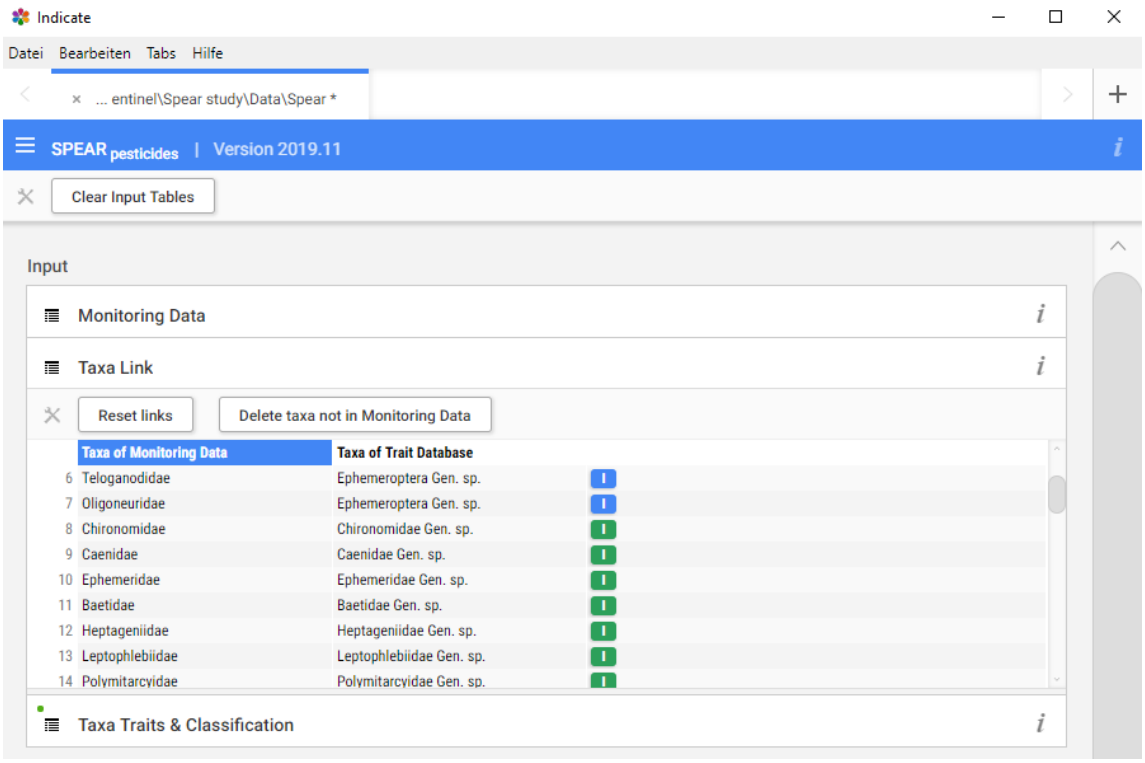
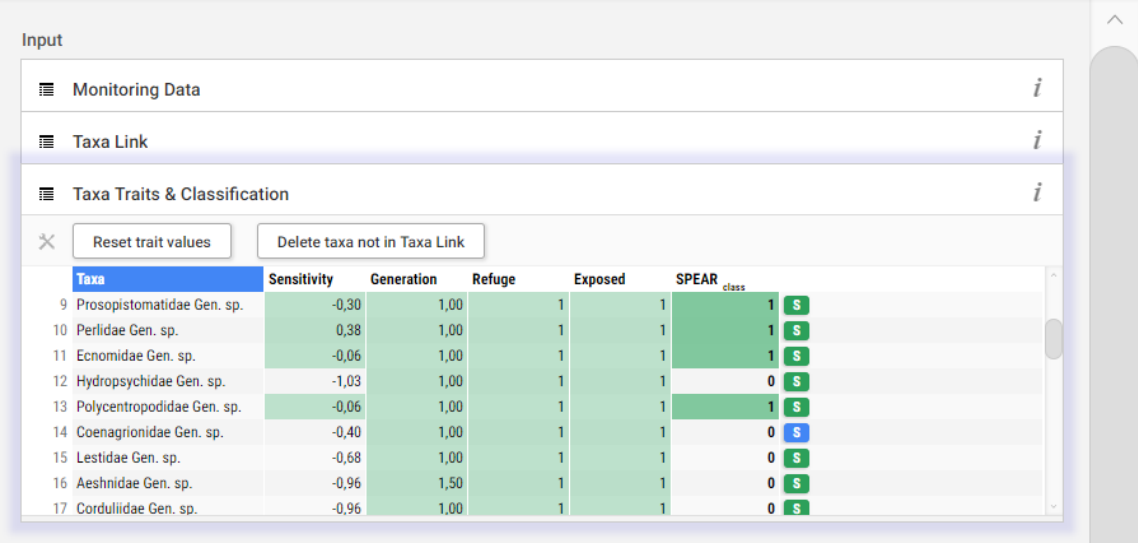


Figure 5.4 The INDICATE program showing the interface where each taxa is linked to its corresponding trait from the database. At this stage, it is imperative to ensure that each taxa recorded is matched with the correct taxa trait. Usually, the program picks the trait of the taxa automatically, or picks the closest relative, but if none is available, then this has to be done manually and should be done by choosing the closest relative or inputting the information manually in the next step.

By default, INDICATE v. 1.2.0 uses a trait data base for the European $SPEAR_{pesticides}$ index v. 2019.11 (Knillmann *et al.,* 2018). In order to use the adapted $SPEAR_{pesticides}$ version for East Africa, open the trait data base under "Taxa Traits and Classification" and repeat the modifications I describe in the results section of the main article: First, set the sensitivity (*s*-value) of *Coenagrionidae* to -0.4, so that the taxon will move from the $SPEAR_{pesticides}$ class 1 ("at risk" to 0 ("not at risk"). Second, change the trait "Exposed" for Corixidae from 0 ("not exposed") to 1 ("exposed"), so that the taxon will move from the $SPEAR_{pesticides}$ class 1 to 0. These modifications are only relevant and possible if the monitoring data contain those taxa; taxa in the trait data base that have not been linked to the monitoring data are hidden under "Taxa Traits and Classification". However, the full trait data base can be viewed (and edited) in the section "Advanced Settings" at the bottom of the menu.



	Taxa	Sensitivity	Generation	Refuge	Exposed	SPEAR class	
9	Prosopistomatidae Gen. sp.	-0,30	1,00	1	1	1	S
10	Perlidae Gen. sp.	0,38	1,00	1	1	1	S
11	Ecnomidae Gen. sp.	-0,06	1,00	1	1	1	S
12	Hydropsychidae Gen. sp.	-1,03	1,00	1	1	0	S
13	Polycentropodidae Gen. sp.	-0,06	1,00	1	1	1	S
14	Coenagrionidae Gen. sp.	-0,40	1,00	1	1	0	S
15	Lestidae Gen. sp.	-0,68	1,00	1	1	0	S
16	Aeshnidae Gen. sp.	-0,96	1,50	1	1	0	S
17	Corduliidae Gen. sp.	-0,96	1,00	1	1	0	S

Figure 5.5 The INDICATE program showing the taxa trait and SPEAR classification being input manually. If the INDICATE program is unable to correctly link the taxa found in the field with any taxa from the database, the information can be manually input at this stage (if known), and a SPEAR classification can be given based on the information input for each taxa. The information for already identified taxa can also be altered here, if the traits from the database do not match up to date information from experiments or more recent literature.

After these modifications, INDICATE automatically calculates the East African SPEAR index according to equation 2:

$$SPEAR_{pesticides} = \frac{\sum_{i=1}^{n}(4x_i+1)\cdot y}{\sum_{i=1}^{n} log_{10}(4x_i+1)}$$

with x_i being the observed number of individuals of taxon i, and y being 1 for those taxa classified at risk and 0 for taxa not at risk. SPEAR values for each site can be viewed under "Effect and Exposure" in the "Results" section, and also exported as .csv file.

Figure 5.6 The INDICATE program showing the results from the previous steps. The output shows each site as it was input, along with a $SPEAR_{pesticides}$ value and a TU estimate, which can be compared to actual TU values. These estimate figures give a good indication of pesticide pollution in rivers.

5.2.8 Conversion of $SPEAR_{pesticides}$ to pesticide toxicity and environmental quality

INDICATE v. 1.2.0 automatically converts $SPEAR_{pesticides}$ values to an estimated pesticide toxicity (in maximum toxic unit, TU_{max}) and to an environmental quality (EQ) class that is related to the ecological status classess according to the EU Water Framework Directive (Beketov *et al.,* 2009). However, TU_{max} and EQ provided in INDICATE are not valid when the East African $SPEAR_{pesticides}$ index is used, because they are calculated based on conversion schemes for the European $SPEAR_{pesticides}$ index (Beketov *et al.,* 2009, Knillmann *et al.,* 2018).

Instead, the East African $SPEAR_{pesticides}$ index may be manually converted to a predicted background toxicity using equation 5 below. This equation has been established using macroinvertebrate and water samples concurrently collected from 16 sites in 13 streams in Western Kenya (see main article). Samples were collected during the main rainy season, but not during flooding events. Therefore, the equation relates $SPEAR_{pesticides}$ values to background toxicity outside run-off events. Studies in European streams showed that long-term pesticide effects on the macroinvertebrate community are typically driven by short-term peak concentrations during run-off events following heavy rainfall (Liess & Schulz 1999, Liess & von der Ohe 2005, Reiber *et al.,* 2020). Peak pesticide concentrations in water during run-off events are approximately four times as high as the background concentrations outside such events in temperate streams of Europe and North America (Kreuger, 1998; Liess *et al.,* 1999; Münze *et al.,* 2017; Schäfer *et al.,* 2008; Williams *et al.,* 1995). Assuming that this relation may also hold in afrotropical streams, peak pesticide exposure during run-off may be estimated from the East African $SPEAR_{pesticides}$ values using equation 6 from the main article:

The button "Effect Plot" in INDICATE provides a graphical overview on the distribution of SPEAR values across the sampling sites. The color code refers to the EQ classes for the European SPEAR index and thus should be ignored when using the East African $SPEAR_{pesticides}$ index. Similarly, the button "Effect Distribution Plots" provides an overview on the distribution of EQ classes and should not be considered for East African samples.

I established no conversion scheme from the East African $SPEAR_{pesticides}$ index to EQ classes because they relate to the Water Frame Work Directive that is only relevant in the European regulatory context. African countries have developed or may develop own criteria for the classification of water quality. As a guideline, pesticide exposure in sampling sites may be classified based on the estimated TU_{max} during run-off as follows:

$\log_{10}(TU_{max}) > -1.5$ -> high pesticide exposure

$-3.5 < \log_{10}(TU_{max}) \leq -1.5$ -> moderate pesticide exposure

$\log_{10}(TU_{max}) \leq -3.5$ -> low pesticide exposure

These thresholds follow observations in European streams that $SPEAR_{pesticides}$ values decrease most strongly in the range between -1.5 and -3.5 to -4.0 $\log_{10}(TU_{max})$ (Knillmann *et al.,* 2018; Schaefer *et al.,* 2012). This trend was also indicated in the Kenyan samples (Fig. 5.8). In the same range of toxicity, leaf litter degradation (Münze *et al.,* 2017; Schaefer *et al.,* 2012), the

exchange of sensitive vs. tolerant species (Reiber *et al.,* 2020) and the development of insecticide resistance (Becker & Liess, 2017, Shahid *et al.,* 2018) responded most strongly to pesticide pollution. When the thresholds are converted from TU_{max} to $SPEAR_{pesticides}$ values using equation 6 from the main article, pesticide exposure may be directly classified from the East African $SPEAR_{pesticides}$ index as follows:

$SPEAR_{pesticides} < 0.9$	-> high pesticide pollution
$0.9 \leq SPEAR_{pesticides} < 1.8$	-> moderate pesticide pollution
$SPEAR_{pesticides} > 1.8$	-> low pesticide pollution

5.2.9 Application of other indicators of freshwater pollution

In order to compare the performance and specificity of $SPEAR_{pesticides}$ in the identification of pesticide pollution, I additionally applied the Biological Monitoring Working Party (BMWP) and the South African Scoring System (SASS5) bioindicators for freshwater quality. In contrast to $SPEAR_{pesticides}$, the BMWP and SASS5 indicators are based on a gradual instead of a binary classification system for the sensitivity of freshwater macroinvertebrates (mainly to low oxygen levels) ranging from 1 to 10. Like $SPEAR_{pesticides,}$ the indicators have been established for freshwater families from a different region, but have been also applied in sub-Saharan Africa beyond their countries of origin (Bere & Nyamupingidza, 2014, Ochieng *et al.,* 2020)

The BMWP score system was developed in the 1980s and refined later to assess organic pollution in British streams (Paisley *et al.,* 2014). For a refined assessment according to the European Water Frame Work Directive, ecological quality indices are derived from the BMWP score by comparing observed vs. expected values based on an elaborate stream classification system. Such a classification system is not available for Kenyan streams; therefore, I simply calculated the average score per taxon (ASPT) using the software ASTERICS – AQUEM/STAR Ecological River Classification System v. 4.0.4, (https://www.gewaesser-bewertung.de/index.php?article_id=419andclang=0). SASS5 was originally developed in 1994 and refined in 2002 to determine the condition or 'health' of rivers in South Africa (Dickens & Graham, 2002). As recommended, I calculated the Average Score Per Taxa (ASPT) using the score tables available in Dickens and Graham (2002).

Finally, I calculated the proportion of ephemeropteran, plecopteran and trichopteran insects on the overall individual number (EPT) and the species diversity (Shannon index) as more

general descriptors of the freshwater macroinvertebrate community. Both descriptors are considered to decrease with increasing levels of water pollution (Knillmann *et al.,* 2018).

Additionally I estimated the run-off potential of a site, i. e. a predictor for the risk of pesticide exposure from surface run-off (Schriever *et al.,* 2007). I used a highly simplified approach by classifying sites on an ordinal scale based on the average local ground slope and the average width of riparian strips up to 2 km upstream as visible from online satellite imagery. Slope was classified as flat, low, medium and high. The run-off potential was then classified as follows: 1 (none): ≥ 30 m buffer strip, or ≥ 20 m buffer strip and flat or low slope; 2 (low): 21 – 30 m buffer strip, or 11 – 20 m buffer strip and flat or low slope; 3 (moderate): 11 – 20 m buffer strip, or 6 – 10 m buffer strip and flat or low slope; 4 (high): ≤ 10 m buffer strip, or ≤ 5 m buffer strip and flat or low slope.

5.2.10 Data analysis

Data analysis was performed using R version 3.6.2. Because $SPEAR_{pesticides}$ has been developed for small and medium streams with flowing water, I limited our analysis to samples from natural streams (no artificial channels) with an average width of < 20 m and an estimated average flow velocity > 1 cm/s (based on the drift-body method). Two sites from a small river close to Kisii (PS42 and PS43) were excluded because biological sampling took part during high water. These samples were characterized by low numbers of taxa and individuals, as well as unusually high pesticide concentrations as compared to the $SPEAR_{pesticides}$ values, suggesting that sampling took place during a run-off event. Therefore, I considered neither the $SPEAR_{pesticides}$ nor the pesticide data from this stream comparable to the other sites. Altogether, our analysis included 16 sites from 13 different streams. Three of the streams were sampled twice, with an average distance of ca. 2 km between both sites. Macroinvertebrate samples and toxic units from sites of the same stream were considerably more similar than those from different streams; to avoid pseudo-replication I therefore aggregated data from different sites of the same stream using the mean values.

The original and the revised $SPEAR_{pesticides}$ index, as well as the associated estimated TU_{max} were compared to the TU_{max} from pesticide measurements using one-way linear regression; TU_{max} was log_{10}-transformed prior to the analyses. To assess the potential disturbance of the sampling sites by additional stressors other than pesticides, I investigated the distribution of the measured TU_{max} and physicochemical water parameter values using violin plots; the data were compared to thresholds for a good ecological status from the literature. To assess the performance and the specificity of $SPEAR_{pesticides}$ for pesticides, I analyzed responses of the

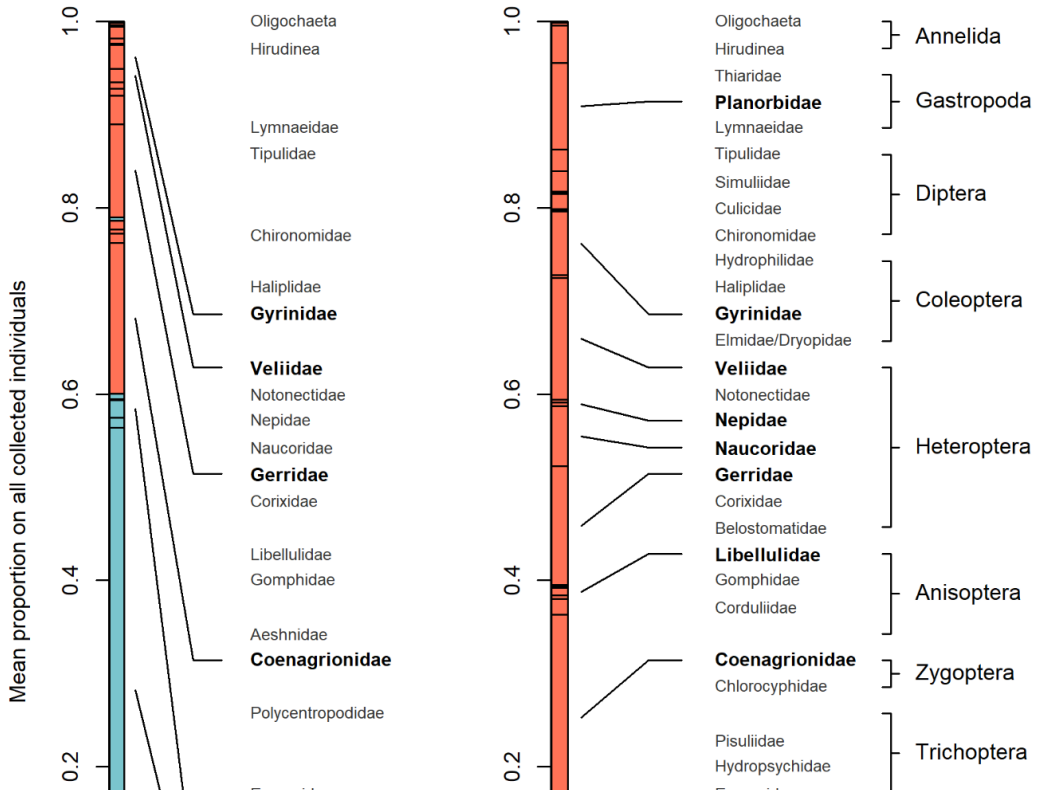
$SPEAR_{pesticides}$ index and of the additional bioindicators and descriptors to the physicochemical parameters using one-way linear regression. Additionally, relations of the $SPEAR_{pesticides}$ index and those physicochemical parameters that had a (marginally) significant effect on the $SPEAR_{pesticides}$, BMWP or SASS5 bioindicator were summarized using a principal component analysis (PCA). Finally, I performed pairwise correlations among the bioindicators and among those environmental variables that significantly affected these bioindicators in order to identify confounding factors.

5.3 Results

5.3.1 Pesticide pollution and effects on the macroinvertebrate community

I observed considerable pesticide toxicity to freshwater macroinvertebrates in our 16 study sites from 13 streams, quantified as the maximum toxic unit derived from chemical water analyses (TU_{max}, see methods). TU_{max} exceeded the threshold range for environmental effects of 10^{-4} to 10^{-3} (Schaefer *et al.,* 2012) in nine and eight streams, respectively. The results indicate that pesticide pollution was a relevant environmental stressor in the study area. Pesticide toxicity was driven by the insecticides diazinon (7 sites, TU = $10^{-2.36}$ – $10^{-1.81}$), pirimiphos-methyl (3 sites, TU = $10^{-1.86}$ – $10^{-1.21}$), bendiocarb (1 site, TU = $10^{-2.54}$) and imidacloprid (1 site, TU = $10^{-3.47}$).

Overall, I identified 35 macroinvertebrate families in the streams. The proportion of taxonomic groups changed markedly with increasing pesticide toxicity (Fig. 5.7). Non- or



marginally polluted sites with $TU_{max} < 10^{-3}$ were dominated by the ephemeropteran families *Baetidae* and *Heptageniidae*, the zygopteran family *Coenagrionidae*, the heteropteran families *Gerridae* and *Veliidae*, and by the coleopteran family *Gyrinidae*. These families occurred in at least half of the low-polluted streams and also showed the highest proportions on the mean number of individuals collected in a low-polluted stream (relative abundances). The same families occurred also in at least half of the highly polluted streams with $TU_{max} \geq 10^{-3}$, but additionally the ephemeropteran family *Caenidae*, the anisopteran family *Libellulidae*, the heteropteran families *Naucoridae* and *Nepidae*, and snails of the family *Planorbidae* were observed in the majority of these streams. As the most prominent changes in the community composition, the mean relative abundance of *Baetidae* decreased from 56 % (range: 7 – 94 %) in lowly polluted streams to 10 % (range: 0.2 – 38 %) in highly polluted streams (n = 5 lowly and 8 highly polluted sites, $\chi^2 = 8.29$, $p = 0.004$ using a binomial generalized linear model). At the same time, *Planorbidae* increased from 0 % to 9 % (range: 0 – 36 %, $\chi^2 = 7.95$, $p = 0.005$) in highly polluted streams. Therefore, changes in the community composition with pesticide pollution were driven by the response of frequently occurring taxa, suggesting that $SPEAR_{pesticides}$ may perform consistently across random samples from different streams. The results are consistent with Reiber *et al.,* (2020) who identified mayflies and snails among those macroinvertebrate taxa that most strongly decrease or increase with pesticide pollution in European streams, respectively.

Figure 5.7: The freshwater macroinvertebrate community composition changes in streams with high pesticide pollution. The stacked bars show the mean proportions of macroinvertebrate families on the overall number of individuals observed in lowly-polluted (maximum toxic unit $TU_{max} < 10^{-3}$) and in highly polluted streams ($TU_{max} \geq 10^{-3}$). The number of individuals ranged from 32 to 154 (mean: 94) in the 5 lowly polluted streams and from 37 to 462 (mean: 152) in the 8 highly polluted streams. Families that were recorded in at least 50 % of the streams are shown in in bold. Taxa shown in blue have been classified as being at risk in the adapted $SPEAR_{pesticides}$ indicator for East African streams, taxa shown in red have been classified not at risk.

5.3.2 Adaptation of $SPEAR_{pesticides}$ to East African streams

Most of the 35 macroinvertebrate families sampled were automatically linked to existing taxa in the trait database provided in the software INDICATE. Only three families did not exist in the database because they do not occur in Central European streams. These families were

manually linked to higher taxa available in the database: *Pisuliidae* was linked to Trichoptera Gen. sp., *Oligoneuridae* was linked to Ephemeroptera Gen. sp., and *Chlorocyphidae* was linked to Zygoptera Gen. sp. *Culicidae* was automatically linked to Diptera Gen. sp., but traits are missing for this taxon (due to a lack of data because dipteran species are too heterogeneous to be aggregated), and they were therefore automatically excluded from the calculation of $SPEAR_{pesticides}$.

Though developed for European streams, the existing $SPEAR_{pesticides}$ index v. 2019.11 from Knillmann *et al.* (2018) showed reasonable correlation with the measured pesticide toxicity (TU_{max}) from chemical water analysis when applied to the Kenyan macroinvertebrate communities, (R^2 = 0.43, Fig. 5.8a). The correlation of the $SPEAR_{pesticides}$ index with toxic pressure increased when I re-classified two taxa based on additional information for Kenyan taxa. First, the damselfly family of *Coenagrionidae* has been classified at risk in the European $SPEAR_{pesticides}$ index v. 2019-11, based on a relatively high physiological insecticide sensitivity (*s*-value = -0.24). This value is only slightly above the threshold for sensitive taxa ($s \geq$ -0.36). The classification was based on toxicity data for the European species *Eschnura elegans*. However, acute toxicity tests with the insecticides diazinon and imidacloprid on freshwater macroinvertebrates collected in the study region of Western Kenya revealed that the tolerance of local coenagrionid species is comparable to those of other taxa such as *Notonectidae*, *Chironomidae* and *Dytiscidae* that have been classified as insensitive (Becker *et al.,* 2020a). Accordingly, I re-classified *Coenagrionidae* as being insensitive (*s*-value = -0.4); consequently, the taxon moved from the category "at risk" to "not at risk".

Second, in $SPEAR_{pesticides}$ v. 2019.11, the heteropteran family *Corixidae* was considered as non-exposed due to a surrounding layer of air (physical lung) that potentially protect individuals from pesticide exposure in the water. However, in acute toxicity tests I observed high insecticide sensitivity of *Corixidae* from the study region (Becker *et al.,* 2020a). Thus, I re-classified *Corixidae* as being exposed, so that the taxon moved from the category "not at risk" to "at risk". Results of toxicity tests with additional 13 taxa collected in the study region from Becker *et al.* (2020a) were in accordance with the existing classification in the $SPEAR_{pesticides}$ trait data base.

With these changes, I were able to adapt the $SPEAR_{pesticides}$ index to East African streams so that it explained the observed TU_{max} considerably better than the European $SPEAR_{pesticides}$ index v. 2019.11 (R^2 = 0.53, Fig. 5.8b). The East African $SPEAR_{pesticides}$ index decreased with increasing measured pesticide toxicity as follows:

Eq. 4: $$SPEAR_{pesticides} = -0.43 * log_{10}(background\ TU_{max}) - 0.02$$

After solving equation 4 for TU_{max}, the East African $SPEAR_{pesticides}$ index may be used to predict background toxicity in streams from the observed macroinvertebrate community composition:

Eq. 5: $$background\ TU_{max} = 10^{\frac{SPEAR_{pesticides} + 0.02}{-0.43}}$$

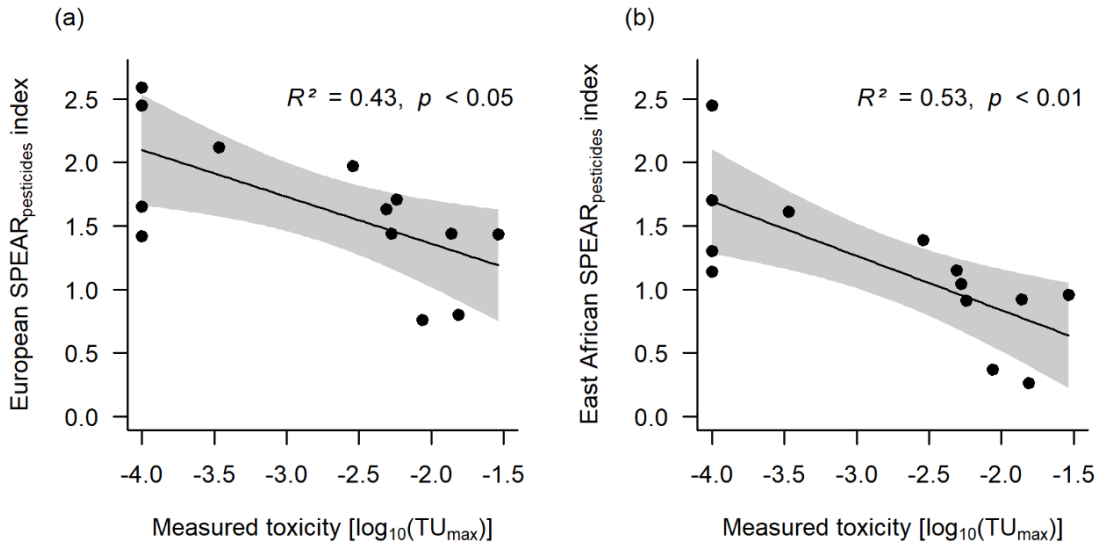


Figure 5.8: $SPEAR_{pesticides}$ indicates pesticide pollution in tropical streams of Western Kenya. Pesticide pollution was quantified as the maximum toxic unit (TU_{max}, restricted to $\geq 10^{-4}$) out of 30 pesticides measured in water samples collected during the rainy season but not during peak exposure. Freshwater macroinvertebrates were identified to the family level. (a) Application of the European $SPEAR_{pesticides}$ index v. 2019.11 from Knillmann *et al.* (2018) to the Kenyan samples; $R^2 = 0.43$, $F = 8.15$, df = 1, res. Df = 11, $p = 0.016$, intercept = 0.62, slope = -0.37. (b) Application of the $SPEAR_{pesticides}$ index after adaption to East African streams. $R^2 = 0.53$, $F = 12.35$, df = 1, res. Df = 11, $p = 0.005$, intercept = -0.02, slope = -0.43.

The mean TU_{max} predicted from equation 5 and the mean observed background TU_{max} coincided well (difference by a factor of < 2.5 or of ≤ 0.4 orders of magnitude (= log_{10}-transformed TU_{max}) across the whole range of observed toxicity, Fig. 5.4a). Across all data points, the ratio of estimated vs. measured TU_{max} ranged from 0.2 to 19.6, i. e. the

predicted toxicity deviated from the measured toxicity by < 1.3 orders of magnitude in all investigated streams.

I related the East African $SPEAR_{pesticides}$ index to background pesticide toxicity measured in water from grab samples collected during the rainy season, but outside run-off events. In contrast, in Europe the $SPEAR_{pesticides}$ index has been related to the short-term peak toxicity measured during run-off events following heavy rainfall (Knillmann *et al.,* 2018; Liess & von der Ohe, 2005; Liess *et al.,* 2008). In temperate streams of Europe and North America, peak pesticide toxicity is approximately four times as high as the background toxicity during the season of main pesticide exposure in early summer (Kreuger, 1998; Liess *et al.,* 1999; Münze *et al.,* 2017; Schäfer *et al.,* 2008; Williams *et al.,* 1995). Assuming that the same ratio of peak vs. background toxicity may hold also in tropical streams, I applied a correction factor of 4 to relate the East African $SPEAR_{pesticides}$ index to an estimated peak exposure during run-off:

Eq. 6: $$estimated\ peak\ TU_{max} = 4 * 10^{\frac{SPEAR_{pesticides} + 0.02}{-0.43}}$$

The response of the East African $SPEAR_{pesticides}$ index to the estimated peak exposure in Kenya could then be compared to the response of the latest European $SPEAR_{pesticides}$ index v. 2019.11 to peak exposure in Germany (Knillmann *et al.,* 2018). The East African $SPEAR_{pesticides}$ index produced considerably higher numbers as compared to the European index, particularly when pesticide toxicity was low (Fig. 5.9b). The more pronounced increase in the East African $SPEAR_{pesticides}$ index with decreasing pesticide exposure indicates differences in the composition of European and East African macroinvertebrate communities and in their response to pesticides. Therefore, the use of a separate conversion scheme from $SPEAR_{pesticides}$ to TU_{max} in afrotropical streams, as established with equation 5 and 6, is justified.

To assess the reproducibility and robustness of the East African $SPEAR_{pesticides}$ index, I extended our data set to all sites sampled in the study area of Western Kenya (see Becker *et al.,* 2020a). The 48 sites from 40 water bodies included habitats for which $SPEAR_{pesticides}$ was not designed such as artificial irrigation channels, rice fields, reservoirs, oxbow lakes and large rivers. I still observed a relation of the East African $SPEAR_{pesticides}$ index with TU_{max} that was very similar to those in Fig. 5.8b, but the non-explained variation increased considerably (R^2 = 0.13, F = 5.55, df = 1, res. df = 38, p = 0.024, intercept = 0.13, slope = -0.31).

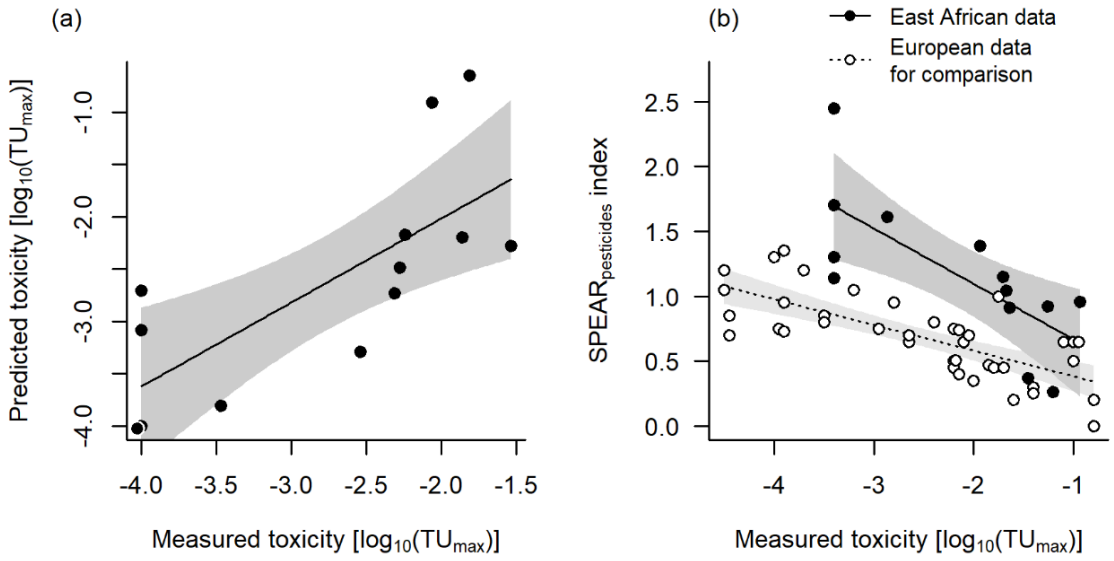


Figure 5.9: Performance of the East African $SPEAR_{pesticides}$ index. (a) Background pesticide toxicity (maximum toxic unit, TU_{max}) predicted with the East African $SPEAR_{pesticides}$ index correlates with the measured background toxicity in Kenyan water samples. $R^2 = 0.54$, $F = 12.97$, df = 1, res. df = 11, $p = 0.004$, intercept = -0.41, slope = 0.80. TU_{max} was restricted to $\geq 10^{-4}$, because lower toxicity could not be measured due to detection limits for pesticides. TU_{max} has been $\log_{10}$-transformed, so that differences are presented in orders of magnitude. (b) Relation of the East African $SPEAR_{pesticides}$ index with the estimated peak pesticide toxicity during run-off in Kenyan streams, as compared to the relation of the European $SPEAR_{pesticides}$ index v. 219.11 with measured peak toxicity in German streams (data from Knillmann *et al.,* 2018). Peak exposure in Kenyan streams was estimated by multiplying the measured background toxicity with a correction factor of 4 (see main text for justification).

5.3.3 Specificity of the East African $SPEAR_{pesticides}$ index to pesticides

To assess the potential impact of confounding factors and the specificity in the response of the adapted East African $SPEAR_{pesticides}$ index to pesticide pollution, I first investigated the potential influence of additional physicochemical parameters on the macroinvertebrate community. A comparison of the observed physicochemical water parameters with values recommended for good water quality from the literature revealed that pesticide pollution was indeed one of the dominant stressors at the sampling sites (Fig. 5.10). Additionally, in many streams I observed very high levels of phosphate and turbidity, as well as low levels of

carbonate hardness and dissolved oxygen, which may have contributed in shaping the macroinvertebrate community.

Next, I assessed the sensitivity of the adapted $SPEAR_{pesticides}$ index for East Africa to such additional stressors. The $SPEAR_{pesticides}$ index decreased significantly not only with increasing pesticide toxicity (measured TU_{max} and TU_{sum}) and run-off potential, but also with carbonate hardness, conductivity, and turbidity (Table 5.1). However, pesticide toxicity was significantly correlated with each of these confounding factors (Table 5.2). All these factors were associated with a dominant environmental gradient identified by the first principal component in a principal component analysis (PCA). This gradient explained 61% of the total variation and likely reflects a range of different stream types (Fig. 5.11). On one end, lowly polluted, fast-flowing mountain streams showing high $SPEAR_{pesticides}$ values (streams nr. 27, 28, 31 and 32) were running in comparably steep valleys through intensely cultivated tea plantations but were protected from run-off by wide buffer strips so that run-off potential and pesticide toxicity was low. On the other end, lowland streams were slowly flowing and showed higher run-off potential due to surrounding agriculture with only small buffer strips. These streams showed high pesticide toxicity, as well as sediment (turbidity) and phosphate pollution that were associated with low $SPEAR_{pesticides}$ values and high conductivity, respectively.

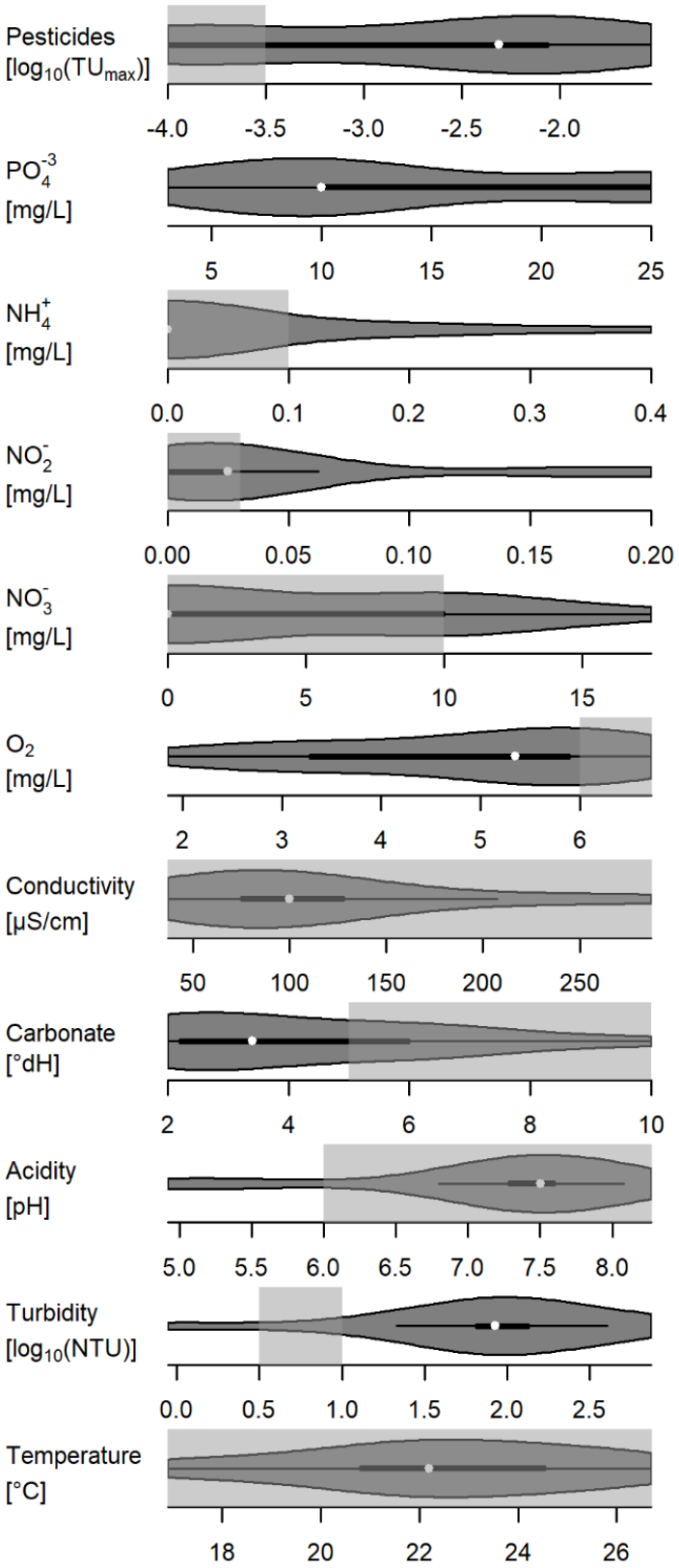


Figure 5.10: Distribution of physicochemical parameter values at the study sites. The violin plot shows the kernel probability density of the data points across the parameter values. White points indicate the median, black boxes the interquartile range, and black lines 1.5 x

the interquartile range. Light-grey boxes indicate the range of values considered typical or recommend for streams with good water quality.

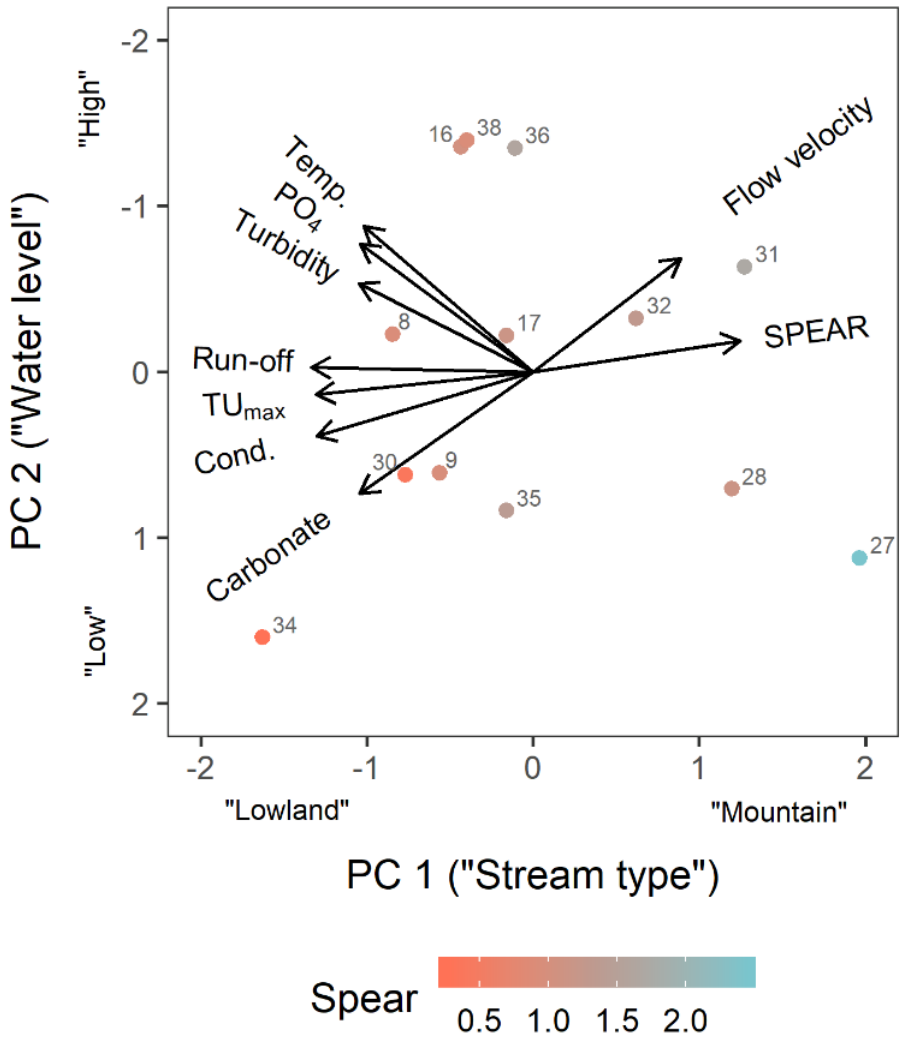


Figure 5.11: Principal component analysis of the East African SPEAR$_{pesticides}$ index and those environmental variables that (marginally) significantly correlated with any of the investigated bioindicators. The first principal component (horizontal axis) explains 60.7 % of the overall variation and was considered to represent a gradient of stream types. The second principal component (vertical axis) additionally explains 14.7 % of the overall variation and was considered to represent a gradient of high water level. Each point represents a stream, colors refer to the average SPEAR$_{pesticides}$ value obtained from each stream.

Though all these confounding stressors increased simultaneously with the first principal component (considered as stream type), they could be disentangled along the second principal component (Fig. 5.11): On one end, I observed slow flowing streams with clear water but high conductivity and carbonate hardness. Streams on the other end showed high flow velocity, turbidity and phosphate concentrations, but low conductivity and carbonate hardness. I consider the second principal component to represent a gradient of raised water level at the day of sampling. Raised water levels may have increased flow velocity, as well as turbidity and phosphate levels due to erosion, and decreased conductivity and carbonate levels due to dilution with rainwater. In contrast, neither run-off potential, nor pesticide toxicity (TU_{max}) or $SPEAR_{pesticides}$ responded to this potential gradient of water levels. First, run-off requires not only high water but also nearby agricultural fields. Second, raised water does not mean flooding in this context, which might have been associated with pesticide pollution from run-off (flooded sites were excluded from the analysis, see methods). The $SPEAR_{pesticides}$ index was thus most closely associated with pesticide toxicity and run-off potential, indicating indeed a high specificity of the adapted $SPEAR_{pesticides}$ indicator for pesticide pollution in Western Kenyan streams. Each of the higher principal components explained only ≤ 8 % of the total variance, and they were thus not further analyzed.

Finally, I compared the performance of $SPEAR_{pesticides}$ in indicating pesticide pollution to those of other bioindicators and commonly applied descriptors of the macroinvertebrate community. Pesticide toxicity most strongly correlated with the East African $SPEAR_{pesticides}$ index (R^2 = 0.53), followed by the European $SPEAR_{pesticides}$ index (R^2 = 0.43) and the average score per taxon (aspt) of the BMWP indicator (R^2 = 0.33, Tab. S1). Correlation with the aspt of the SASS5 indicator was lower (R^2 = 0.19) and not significant. In contrast to $SPEAR_{pesticides}$ that was only related to pesticides and their associated stressors (see above), the BMWP and SASS5 indicators were additionally related to phosphate pollution and temperature but did not significantly correspond to run-off potential. The EPT index (cumulative proportion of *Ephemeroptera*, *Plecoptera* and *Trichoptera*) showed a significant but low negative response to pesticide pollution (R^2 = 0.10), turbidity and carbonate hardness, but increased with flow velocity. The Shannon index for species diversity increased with pesticide pollution, but the response was only marginally significant and may relate to the generally concurrent increase in species richness and pesticide pollution from the spring to more downstream sections (Minshall *et al.,* 1985). The different bioindicators correlated with each other. The East African $SPEAR_{pesticides}$ index increased with the aspt of the BWMP and SASS5 indicator and with the EPT index, but decreased with species diversity (Tab. S2). BMWP and SASS5 were

clearly correlated with each other, but not with the EPT index and species diversity. The EPT index decreased with increasing species diversity.

5.4 Discussion

I adapted the $SPEAR_{pesticides}$ bioindicator for the quantification of pesticide exposure to afrotropical conditions and demonstrated its use in thirteen Kenyan streams. In the following, I discuss the performed modifications of $SPEAR_{pesticides}$ and the results from the present case study.

5.4.1 Pesticide pollution in Western Kenya

Pesticides are an important stressor to freshwater macroinvertebrates in small and medium streams of western Kenya. Our results confirm earlier conclusions from various freshwater habitats of the same study area (Kandie *et al.*, 2020a); in that study, the chemical freshwater pollution was assessed and the ecological risk (TU_{sum}) identified was highest for macroinvertebrates due to insecticide exposure. Pesticide toxicity observed in streams of the present study was comparable to those in European landscapes characterized by intensified agriculture (Becker *et al.,* 2020b). In three out of 13 streams, I observed toxic units exceeding the threshold of 10^{-2} that is considered safe according to the first tier of governmental risk assessment in the European Union (EFSA, 2013). The results illustrate a need for the monitoring and regulation of pesticide application in order to reduce pesticide exposure in freshwater. In Kenya, plant protection products are sold at relatively low prices without the need for a certificate of competence from retailers, making them widely available to small farmers who are then not informed of the necessary precautions needed to comply to the proposed environmentally safe use. This includes products containing active substances that have been banned in many high income countries, such as the non-selective insecticide diazinon that can be applied on a wide range of crops (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/) and was driving toxicity in most of our sampling sites.

5.4.2 Adaptation of $SPEAR_{pesticides}$ to East African streams

Effects on the macroinvertebrate community composition were successfully quantified even with the non-modified $SPEAR_{pesticides}$ indicator v. 2019.11 developed for Europe. $SPEAR_{pesticides}$ values decreased approximately log-linearly with increasing pesticide toxicity when the maximum toxic unit (TU_{max}) ranged from 10^{-4} to $10^{-1.5}$. At lower TU_{max} values

$SPEAR_{pesticides}$ leveled off, confirming a threshold of 10^{-4} to 10^{-3} TU_{max} below which no effects on freshwater macroinvertebrates have been observed (Schaefer *et al.,* 2012).

Reclassification of the families *Coenagrionidae* and *Corixidae* based on toxicity tests with Kenyan species (Becker *et al.,* 2020a) improved the correlation of $SPEAR_{pesticides}$ with TU_{max}. I suggest determining whether such a reclassification may increase the link between toxicity and invertebrate community composition also in European streams. $SPEAR_{pesticides}$ values from both the European and the adapted East African index were considerably higher in Kenya than those observed in Central European streams (Knillmann *et al.,* 2018), particularly when pesticide toxicity was low. I speculate that in East African streams, the natural proportion of non-vulnerable taxa may be lower. Indeed, macroinvertebrate communities in most Central European streams are dominated by amphipod crustaceans of the genus *Gammarus sp.,* and by mayflies of the *Baetis rhodani* / *vernus* group (Becker & Liess, 2017). Both groups are considered non-vulnerable to pesticides and often contribute to more than 50 % of individuals. In contrast, amphipod crustaceans were missing in Kenya, confirming previous observations e. g. from Elias *et al.* (2014). Additionally, I classified mayfly families observed in Kenya as vulnerable (confirmed by a strong decrease with increasing pesticide toxicity, and consistent with most other, less abundant European mayflies). Hence, $SPEAR_{pesticides}$ values in East Africa are associated to higher levels of pesticide toxicity than similar $SPEAR_{pesticides}$ values in Europe, and I adapted the link between the East African $SPEAR_{pesticides}$ index and TU_{max} accordingly.

Using the East African $SPEAR_{pesticides}$ index, the measured pesticide toxicity (TU_{max}) could be predicted with a precision of 1.3 orders of magnitude in each of the streams. This variation covered not only uncertainties in the $SPEAR_{pesticides}$ approach, but also in the measurement of pesticide concentrations and in their conversion to toxic units. Considering the generally high levels of variability and uncertainty in ecotoxicology, as reflected by an assessment factor of 100 (2 orders of magnitude) in first tier European risk assessment (EFSA, 2013), results from chemical analyses and from the application of $SPEAR_{pesticides}$ coincided reasonably well.

5.4.3 Specificity of $SPEAR_{pesticides}$ for effects of pesticides

The overall variability in values of the East African $SPEAR_{pesticides}$ index was explained to 53% by the measured pesticide toxicity, and to 45% by the estimated run-off potential based on catchment slope and width of buffer strips. The estimated run-off potential was also closely associated with pesticide toxicity and thus provides a fast and simple method for the identification of potential sampling sites of interest, and for the verification of calculated

$SPEAR_{pesticides}$ values: Sites where the estimated pesticide toxicity based on $SPEAR_{pesticides}$ does not fit to the estimated run-off potential may be heavily affected by additional stressors and should be investigated further.

Additional stressors that affected the East African $SPEAR_{pesticides}$ index included carbonate hardness, conductivity and turbidity. All these confounding factors increased with pesticide toxicity along a gradient of different stream types but could be disentangled along a second, independent gradient of increasing water levels. While carbonate hardness, conductivity and turbidity varied with water levels presumably due to erosion and dilution, the $SPEAR_{pesticides}$ index, pesticide pollution and run-off potential did not, because raised water levels alone do not increase run-off without nearby agricultural fields. Thus, our results show that indeed pesticide toxicity and not confounding factors is driving the $SPEAR_{pesticides}$ index (Table 5.1, 5.2 & 5.3).

In contrast to $SPEAR_{pesticides}$, other bioindicators for the assessment of freshwater quality have not been designed to specifically indicate effects of pesticides (Dickens & Graham, 2002; Paisley *et al.,* 2014). As expected, the BMWP and SASS5 scoring system therefore responded to a broader range of stressors including phosphate and sediment pollution that may be associated with oxygen depletion. Similarly, the EPT index and the Shannon index for species diversity most strongly responded to stressors other than pesticides.

Our application of $SPEAR_{pesticides}$ is not the first attempt to establish or apply bioindicators for the assessment of freshwater quality in East Africa (Masese *et al.,* 2009; Ochieng *et al.,* 2020; Shimba & Jonah, 2016). These case studies illustrate the growing interest in the use of bioindicators for freshwater monitoring but did not explicitly consider effects of a specific stressor such as pesticide pollution. Apart from our study, the only application of $SPEAR_{pesticides}$ in sub-Saharan Africa I are aware of has been described in Malherbe *et al.* (2018). The authors applied a previous version of the European $SPEAR_{pesticides}$ index and the Australian $SPEAR_{pesticides}$ index (Schaefer *et al.,* 2011a) to macroinvertebrate samples from the Crocodile River and the Harts River in South Africa. The sampling sites were located upstream, adjacent to and downstream of two large irrigation schemes. The $SPEAR_{pesticides}$ index decreased with increasing estimated pesticide toxicity ($R^2 = 0.26$) but the correlation was not significant.

Table 5.1: Linear one-way regression of bioindicators of freshwater pollution vs. environmental parameters. Pesticide pollution was quantified as TU_{max}, TU_{sum} and as runoff potential (based on slope and buffer strips); flow velocity (Flow) is shown in m/s; NH_4, NO_{2-}, NO_{3-}, PO_4 and dissolved oxygen (O_2) in mg/L; temperature in °C; conductivity in µS/cm; carbonate hardness (CH) in °dH and turbidity (Turb.) in NTU. Flow, NH_4, NO_{2-}, NO_{3-}, PO_4, conductivity, O_2, CH and turbidity were ln-transformed prior to analysis. Freshwater macroinvertebrates were identified to the family level. For the BMWP and SASS5 indicator, the average score per taxon (aspt) was calculated. n = 13, df = 1 and residual df = 12 in all analyses. Significant results ($p < 0.05$) are shown in bold, marginally significant results ($0.05 \leq p < 0.1$) in gray.

	$SPEAR_{pesticides}$ (revised)				**$SPEAR_{pesticides}$ v. 2019.11**				**BMWP (aspt)**			
	R²	***F***	***p***	**Slope**	***R²***	***F***	***P***	**Slope**	***R²***	***F***	***p***	**Slope**
TU_{max}	**0.53**	**12.35**	**0.005**	**-0.43**	**0.43**	**8.15**	**0.016**	**-0.37**	**0.33**	**5.49**	**0.039**	**-0.27**
TU_{sum}	**0.52**	**11.96**	**0.005**	**-0.41**	**0.43**	**8.16**	**0.016**	**-0.35**	**0.35**	**5.85**	**0.034**	**-0.27**
Runoff	**0.45**	**9.09**	**0.012**	**-0.38**	**0.38**	**6.75**	**0.025**	**-0.34**	0.20	2.70	0.129	-0.20
Flow	0.22	3.12	0.105	0.26	0.30	4.74	0.052	0.29	0.08	0.91	0.360	0.12
NH_4^+	<0.01	0.01	0.910	-0.03	<0.01	0.04	0.836	-0.06	0.01	0.06	0.811	-0.06
NO_2^-	0.09	1.14	0.309	-0.19	0.06	0.70	0.420	0.22	0.73	0.73	0.412	-0.12
NO_3^-	0.03	0.39	0.547	0.17	0.06	0.72	0.415	-0.22	0.01	0.13	0.722	-0.08
PO_4^{3-}	0.21	2.95	0.114	-0. 43	0.04	1.87	0.171	-0.25	0.30	4.66	0.054	-0.41
Temp.	0.17	2.32	0.156	-0.08	0.05	0.60	0.455	-0.04	0.24	3.45	0.090	-0.08
CH	**0.36**	**6.06**	**0.032**	**-0.75**	0.30	4.67	0.054	-0.66	**0.40**	**7.47**	**0.019**	**-0.65**
Cond.	**0.56**	**14.27**	**0.003**	**-0.83**	**0.47**	**9.71**	**0.010**	**-0.73**	**0.54**	**12.69**	**0.004**	**-0.65**
pH	0.20	2.69	0.129	0.26	0.20	2.76	0.125	0.26	0.03	0.33	0.576	0.08
O_2	0.06	0.68	0.427	0.44	0.12	1.55	0.239	0.61	0.11	1.42	0.259	0.49
Turb.	**0.42**	**8.07**	**0.016**	**-0.23**	0.29	4.56	0.056	-0.18	0.25	3.73	0.080	-0.14

	SASS5 (aspt)				**EPT[a]**				**Shannon**			
	R²	***F***	***p***	**Slope**	***R²***	***X²***	***P***	**Slope**	***R²***	***X²***	***p***	**Slope**
TU_{max}	0.19	2.63	0.133	-0.56	**0.10**	**6.62**	**0.010**	**-0.83**	0.23	3.33	0.095	0.27
TU_{sum}	0.19	2.57	0.137	-0.53	**0.10**	**6.37**	**0.012**	**-0.79**	0.22	3.11	0.106	0.25
Runoff	0.20	2.69	0.129	-0.54	0.09	3.50	0.061	-0.73	**0.31**	**4.90**	**0.049**	**0.30**
Flow	<0.01	<0.01	0.951	0.02	**0.15**	**4.90**	**0.027**	**0.97**	0.10	1.21	0.295	-0.17
NH_4^+	0.01	0.08	0.786	0.18	0.01	0.44	0.509	-0.43	0.25	3.67	0.082	0.47
NO_2^-	0.01	0.12	0.734	-0.14	0.01	0.43	0.513	-0.27	0.16	2.09	0.176	0.23
NO_3^-	0.01	0.17	0.691	-0.24	0.06	2.21	0.137	0.98	0.08	0.97	0.347	-0.25
PO_4^{3-}	0.29	4.52	0.057	-1.09	<0.01	0.01	0.915	-0.08	<0.01	<0.01	0.972	-0.01
Temp.	0.28	4.35	0.061	-0.22	0.01	0.17	0.685	-0.06	<0.01	<0.01	0.999	<0.01
CH	0.16	2.05	0.180	-1.07	**0.10**	**4.86**	**0.028**	**-1.76**	0.23	3.33	0.095	0.57
Cond.	**0.32**	**5.08**	**0.046**	**-1.33**	0.07	3.16	0.076	-1.27	0.21	2.89	0.117	0.48
pH	0.22	3.10	0.106	0.60	<0.01	0.11	0.740	0.13	0.10	1.16	0.304	-0.17
O_2	<0.01	0.05	0.827	-0.26	0.05	1.39	0.238	1.72	0.02	0.22	0.649	-0.24
Turb.	**0.34**	**5.72**	**0.036**	**-0.44**	**0.19**	**6.23**	**0.013**	**-0.70**	0.20	2.77	0.124	0.15

[a]Effects on the proportion of ephemeropteran, plecopteran and trichopteran taxa (EPT) were analyzed using a quasibinomial generalized linear model with a logit link function and the

number of taxa as weights; χ^2 from likelihood ratio tests and McKelvey and Zavoina's pseudo-R^2 are shown.

It should be noted that Malherbe *et al.* (2018) used a very coarse toxicity estimation based on the sampling site location, assuming that pesticide pollution increases from upstream to downstream of the irrigation system. Toxicity estimation thus did not consider pesticide input from outside the irrigation scheme, whereas our data show that subsistence farming may considerably contribute to pesticide pollution. Therefore, the poor performance of $SPEAR_{pesticides}$ in Malherbe *et al.* (2018) may be partly related to uncertainties in the assessment of pesticide exposure. Additionally, the authors sampled macroinvertebrates from large rivers, whereas $SPEAR_{pesticides}$ has been developed for small to medium streams. When I tested the East African $SPEAR_{pesticides}$ index with a larger data set of unsuitable habitats, my conversion scheme from $SPEAR_{pesticides}$ values to pesticide toxicity turned out to be principally robust despite the small sample size used for development. However, the unexplained variance considerably increased, illustrating the importance to consider that the applicability of $SPEAR_{pesticides}$ is limited to small and medium streams with flowing water and no heavy streambed degradation (Liess & von der Ohe 2005).

Table 5.2: Correlations among those environmental variables that influenced $SPEAR_{pesticides}$ or other bioindicators. Significant results ($p < 0.05$) are shown in bold, marginally significant results ($0.05 \leq p < 0.1$) in gray. $n = 13$, df = 11 for all correlations. Carbonate hardness (CH), flow velocity (Flow), conductivity (Cond.), phosphate concentration (PO_4^{-3}) and turbidity (Turb.) were ln-transformed prior to analysis.

	TU_{max}			Runoff potential			Carbonate hardness			Conductivity		
	r	***t***	***p***	***r***	***t***	***p***	***r***	***t***	***p***	***r***	***t***	***p***
Runoff	**0.70**	**3.25**	**0.008**	-	-	-	-	-	-	-	-	-
CH	**0.67**	**2.98**	**0.013**	**0.56**	**2.24**	**0.047**	-	-	-	-	-	-
Cond.	**0.76**	**3.85**	**0.003**	**0.76**	**3.82**	**0.003**	**0.85**	**5.27**	**<0.001**	-	-	-
Turb.	**0.67**	**2.99**	**0.012**	**0.62**	**2.62**	**0.024**	0.34	1.21	0.252	0.38	1.35	0.204
PO_4^{-3}	0.49	1.84	0.092	0.55	2.17	0.053	0.28	0.97	0.354	**0.58**	**2.35**	**0.039**
Flow	-0.55	-2.19	0.051	**-0.63**	**-2.66**	**0.022**	-0.44	-1.65	0.128	-0.55	-2.16	0.054
TU_{sum}	**1.00**	**61.44**	**<0.001**	**0.69**	**3.14**	**0.009**	**0.66**	**2.89**	**0.015**	**0.75**	**3.79**	**0.003**

Table 5.3: Correlations among the applied bioindicators for freshwater pollution. For the BMWP and SASS5 indicator, the average score per taxon (aspt) was calculated. Significant results ($p < 0.05$) are shown in bold, marginally significant results ($0.05 \leq p < 0.1$) in gray. $n = 13$, df = 11 for all correlations.

	$SPEAR_{pesticides}$ (revised)			BMWP (aspt)			SASS5 (aspt)			EPT		
	r	*t*	*p*	*r*	*t*	*p*	*R*	*t*	*p*	*r*	*t*	*p*
BMWP	**0.70**	**3.25**	**0.008**	**-**	**-**	**-**	-	-	-	-	-	-
SASS5	**0.77**	**4.03**	**0.002**	**0.77**	**4.00**	**0.002**	-	-	-	-	-	-
EPT	**0.85**	**5.40**	**<0.001**	0.40	1.44	0.176	0.43	1.59	0.140	-	-	-
Shannon	**-0.73**	**-3.59**	**0.004**	-0.30	-1.05	0.315	**-0.51**	**-1.96**	**0.076**	**-0.82**	**-4.68**	**0.001**

5.5 Conclusions

As shown, the high impact of pesticides on freshwater organisms is not limited to regions with intensified commercial agriculture. Widespread pesticide pollution in Western Kenyan streams and the associated decline in vulnerable macroinvertebrates indicate an ecological risk also in areas dominated by subsistence farming. Potential negative effects on species diversity and on ecosystem services such as leaf-litter degradation and biological pathogen control illustrate the need to improve the risk management of pesticides also in developing countries. Monitoring is essential in this respect to identify hot spots of pesticide pollution for the targeted development of mitigation measures, and to evaluate the effectiveness of actions that have been taken. I adapted the $SPEAR_{pesticides}$ bioindicator for the quantification of pesticide exposure in streams of East Africa. This tool provides a cost-efficient alternative to the complex sampling and analysis of chemicals and thus may facilitate large scale monitoring with limited resources in developing countries.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

This study presents the first evidence of pesticide pollution being a predictor of snail presence and density from a field survey. The analysis of community composition in the field sites highlighted that pesticide pollution was associated with an increase in *Schistosoma*-host snails and a reduction in their competitors in the grazing trophic levels which were more susceptible to pollution. The study also serves as the first evidence of an agrochemical affecting snail density through the alteration of competitor density, whereas previous studies have focused on predator effects (Bajet *et al.,* 2012; Halstead *et al.*, 2018; Halstead *et al.*, 2015; Satapornvanit *et al.*, 2009). Once the effect on the host had been established, the effect on the parasite had to be investigated. The effects of imidacloprid and diazinon, as had never been studied on the host snails *Biomphalaria pfeifferi* and *Bulinus africanus*, had also not been studied on the parasite *Schistosoma mansoni* nor *Schistosoma haematobium.* Similar work has been done by Jones *et al.,* (2020), though on the compounds cypermethrin, deltamethrin, dimethoate and methamidophos. Thus, to address this knowledge gap, the parasites' sensitivity to imidacloprid and diazinon was studied. *Schistosoma mansoni* appeared to be insensitive to imidacloprid and diazinon, with effective concentrations (EC_{50}) higher than those of competitors of host snails such as the ephemopteran genus' Baetidae and Caenidae. This means that should these pesticides enter the natural water systems, the concentrations that would be detrimental to the competitors of the snails such as Baetidae or Caenidae, whose would not affect either the host snails *B. africanus* and *B. pfeifferi*.

The studies conducted on the parasite (chapter 4) determined that the concentrations of the two pesticides needed to immobilise or kill the free-living life-stages of *Schistosoma* were higher than those needed to kill the competitors of the snails such as Baetidae or Caenidae. As such, there is a scenario where the concentrations of pesticidal compounds entering the natural water bodies can boost the host snail population by reducing their competitors. At the same time these concentrations would also allow the miracidia and cercariae to survive and continue transmission. While LC50s give a good indication of the concentrations that would cause a definite effect through immobilizations and death, sublethal doses in which community effects can be observed are known begin at concentrations three orders of magnitude lower than their LC_{50} (Beketov & Liess 2005; Liess & Schulz, 1996). Thus, experiments conducted to observe these effects showed that the miracidia infections reduce

with sublethal doses of both pesticides. However, once within the snail, repeat exposure to these concentrations did not affect the proportion of snails shedding mature cercariae. Therefore, while miracidia may have reduced infections with pesticide pollution, there may not be an overall reduction in transmission as other stages are not affected. Thus, as the few miracidia that are able to get to the snail would be able to continue transmission. From these studies, it must be cautioned that certain compounds harbour the possibility of increasing transmission, and thus risk assessment on the disease should consider this new information. This revelation corroborates a wealth of literature on the matter now, succinctly summarised (Jones *et al.,* 2020), whose analysis of the literature of the effect of agrochemicals on schistosomiasis shows, through simulations, that pollution may lead to increased rates of schistosomiasis. Their analysis draws on a wealth of data which show many possible interactions that can occur but ultimately what will be observed in the field will depend on local agrochemical practices and frequencies.

The studies presented here highlighted that chemical analysis of water samples from rivers take considerable time and resources. The period from initial field sampling to identification of all chemicals from the water was close to two years, and required highly specialised equipment (Kandie *et al.*, 2020a). The cost, time, specialised skill and materials needed present a major deterrent to countries wanting to implement monitoring of environmental pollution as low-income countries lack the income to fund the detection of compounds. An alternative to expensive chemical analysis, is the use of bioindicators which can rapidly assess the ecological effects of pesticides through real-time analysis of macro-invertebrate sampling and community assessment (Liess & Von Der Ohe, 2005). Several indicators exist, however, such as the South African Scoring System (SASS5) (Dickens & Graham, 2002) or the ephemeropteran, plecopteran and trichopteran (EPT) index (Lenat, 1988) are tuned to detect organic pollution. The SPEARpesticide on the other hand, was the first bio-indicator developed for detecting pesticide pollution (Liess & Von Der Ohe, 2005). First developed in Germany, the concept proved successful over two decades across Europe (Liess *et al.,* 2008) before it was adapted to Argentina in South American (Hunt *et al.*, 2017).

Here, the first successful application of SPEARpesticide in Africa was conducted and reported (Chapter 5), showing that the bio-indicator is able to estimate the level of pesticide pollution in small rivers by analysing the macroinvertebrate community composition. The study confirmed the sensitivity of the macro-invertebrates found in the western Kenyan region were similar enough to those found in Europe, such that only two changes needed to be made to the bioindicator to calibrate it to the insects found in tropical Africa. Thus, the

study serves as a proof of concept on the use of SPEARpesticide in Kenya. Whether it can be applied to the wider East and Southern African region will need further investigation and potential calibrations. SPEARpesticide indicator can be a tool to rapidly assess streams for toxicity with a fraction of the costs in order to estimate the pesticide pollution occurring and apply the necessary countermeasures (described in recommendations below). In relation to schistosomiasis, the newly calibrated SPEARpesticides analysis will allow for rapidly assessing the pesticide pollution in a potential snail habitat during disease mapping, and will allow epidemiologists and ecotoxicologists to work together to consider the pollution levels as a risk factor determining the presence of snail hosts in the future. The chapter also deals with the fact that there are physiological differences between taxa from Europe and their relatives in Africa. The differences in sensitivities have not yet fully been explored, and therefore are yet to be explained.

Thus, this thesis introduces the capabilities for interventions of schistosomiasis to account for the risk based on pesticide pollution in habitats in endemic areas, and consider the occurrence of pesticide pollution as a possible risk factor for the presence of host snails and thus, transmission of disease. Further countermeasures would include increasing disease-surveillance through methods such as habitat monitoring (Tchuem Tchuenté, 2017) through evaluation of water bodies for the presence of snails or environmental DNA (eDNA) (Sengupta *et al.*, 2019) from target species that would indicate risk (Stothard *et al.*, 2017). However, this should be complemented with the use of the SPEARpesticide bio-indicator to evaluate future disease carrying potential of the habitats.

The study recommends that the general public be aware of the trade off in using pesticides on crops when there are indirect consequences yet to be uncovered. The evidence in this study should be of particular interest to the health sector, as schistosomiasis has been tabled for elimination by 2030 by the WHO. Thus, if Kenya is to meet this deadline, the ecology of the snail must be understood, and pesticides are currently the most ecologically disruptive occurrence of the past few decades. Therefore, it was expected that it could make an impact on the snail ecosystem, and as evidence shows it could benefit the disease. Disease ecologists also would be privy to this evidence when considering the distribution of snails, and using the bioindicator tested in this study, potential future hotspots of transmission could be identified by locating areas of high pesticide pollution and monitoring the snail-macroinvertebrate community. Policy makers may want to consider indirect effects of pesticide compounds before allowing them into the market."

6.1 Lay summary

Pesticide pollution has proven itself to be a chronic problem with new consequences being discovered regularly. Despite this, their use shows little signs of slowing due to the growing population and unavailability of alternatives that can prevent pests as effectively. Recent work into cheaply monitoring pesticide pollution in Germany led to the development of a bio-indicator named SPecies At Risk (SPEAR)pesticides which was aimed to estimate pesticide pollution levels in streams through macroinvertebrate sampling of the streams and using this community assemblage – pesticide pollution could be estimated. To correctly estimate the pollution, the sensitivities of various freshwater organisms to pesticides had to be tested in the laboratory, and during these test it was discovered that snails from the family Planorbidae were the most tolerant to pesticides. This raised alarms as this family contained snails that hosted the freshwater disease-carrying worms that cause schistosomiasis. Hence, it was hypothesized that pesticide pollution in a natural setting may favour the disease by favouring the host snails tolerant to pesticides, while wiping out competitors and predators. A study was chosen to be conducted in Kenya due to the high rates of schistosomiasis along Lake Victoria, and with the smallest proportion of the shoreline among the three countries that share the lake, this would maximise the chance of getting the snails while also reducing the size of the study area to find them. The study area focused various altitudes in close proximity due to the geological phenomenon, the Rift Valley, which has since allowed the residents to capitalise on the broad range of climates to grow a variety of crops. This also factored into choosing the area as a study site as the different crops would be sprayed with different chemicals, which would allow us to test whether the different blends could also have an effect.

To begin testing the hypothesis, first the facts for which the study was based needed confirming – the host snails are more tolerant to pesticides than their insect counterparts. As the first experiments were conducted in Germany, the tolerance of various freshwater organisms may vary in the different geographical area. So, acute toxicity tests were conducted, using two easily obtained compounds in the region, one neo-nicotinoid and one organophosphate, two of the biggest group of pesticide compounds used globally, and locally. Our tests on 21 different species showed that the host snails were the most tolerant to both pesticides, just like their European counterparts. Thus, the hypothesis was further investigated in the field, 48 sites were selected across the Kenyan shore of Lake Victoria, across seven counties with various crop types and landscapes. As the ecology of the snail's habitat was still

not so well known, with all water bodies such as dams, ponds and rivers expected to have the host snails; thus, all the above habitat types were selected as study sites. Sites were also selected based on crop grown and growing intensity, such that commercial farms such as rice, tea, or sugarcane plantations or in subsistence farms with small-scale maize fields, were all considered. The study found that host snails were prevalent in small streams rather that large rivers or stationary water bodies. More importantly, host snails were found where there was at least a moderate level of pollution, defined by a toxic unit level of above -3, which equates to what would be $1/100^{th}$ the lethal concentration to a reference organism, usually *Daphnia magna*. The field data on the community make-up showed that in these polluted rivers, the proportion of predators remained the same, yet the prey available was limited to host snails. It was therefore concluded, being that the host snails are more tolerant than their competitors, the lack of competitors in polluted field sites indicates that host snails were surviving the pollution better than their competitors. The predators were less affected likely due to their migration capabilities and abundance of prey, which only differed in its shift to more host snails. This increase in host snails in polluted waters increases the risk of transmission as the *Schistosoma* parasite has more opportunities to spread.

The *Schistosoma* parasite is itself at risk most times due to its small size and limited energy resources. It has two life-stages where it is vulnerable as it seeks out its host as microscopic larval forms. It also spends a considerable amount of time inside the intermediate host, the snail, which is aquatic and as shown above, able to thrive in pollution. Therefore, the question raised was whether the parasite could survive the concentrations beneficial to its host. The free-swimming stages were tested for their tolerance against Imidacloprid and Diazinon, which was shown to kill off competitors of the host snails. The free-living larval stages, the miracidia, which seeks out the snail host, and the cercariae, which seeks out the human, both have short-lived life expectancies which can be drastically reduced by pesticide pollution. However, the concentration considered detrimental to these larval stages was found to be higher than that of the competitors of snails. This worrisome fact means that concentrations that could wipe out the snail's competitors and increase the host densities, would not kill of the parasite. The experiments above also investigated whether the miracidia, if left swimming in pesticide, would still be capable of carrying out biological functions such as host-seeking and penetration. This snail-seeking stage, when exposed to non-lethal concentrations for up to six hours, were still able to seek out their host snails and infect them. Lastly, the final experiment showed that snails, once infected and then placed in pesticides, still developed the

human-infecting larval stage. This showed that the parasite maturation within the snail is not interrupted by the chemical build-up of pesticides when the snail hosting the parasite is submerged in pesticides over the course of the five weeks it is estimated that the parasite needs to mature. Thus, all evidence points towards pesticides not hindering transmission as none of the life-stages experimented on showed enough effects to suggest environmentally relevant concentrations would impact the parasite in its regular functioning.

Given that pesticide pollution was first shown to benefit the host snails, by wiping out competitors to the snails in the natural environment, and that the parasite is not too disrupted in its functioning to carry on transmission, I concluded that schistosomiasis transmission is likely to increase with pesticide use due to increase in hosts. To counter this upcoming change in host epidemiology, a bio-indicator was tested for its sensitivity to pesticides to cost-effectively assess the pesticide pollution in the streams of Kenya. As pesticides can be difficult and expensive to detect and quantify in the field, not to mention that the equipment necessary is not easily found within the country. For these reasons, a bioindicator is preferred as it can estimate the levels of pollution using only information of the stream's insect numbers. As such, the estimates can be made rapidly and require much less technology and technical expertise. The SPEARpesticide indicator, developed in Germany was the first indicator tuned specifically to pesticide pollution. This bioindicator needed to be calibrated as the information that it was based on was the sensitivities of freshwater insects of Germany. Therefore, I conducted similar acute toxicities on the insects of western Kenya to validate the sensitivity information. While the tests confirmed most insects' sensitivities were identical between the regions, a couple of adjustments allowed for a new SPEARpesticide bioindicator to be programmed towards the tropical African region. This simple computer program will from now allow for toxicity in rivers of East Africa to be estimated through macroinvertebrate sampling over 30 minutes per river, allowing for rapid assessment of pesticide pollution. This should also allow disease-ecologists to determine the future potential host habitats and disease hotspots.

6.2 Limitations of the study

The field data was collected over one sampling period which was conducted from September to October 2017. Thus, the data captured was limited to sample of the information available and data on seasonal or monthly variations could not be captured. Future research in the area

should therefore be directed at addressing this gap and understanding the temporal differences in macroinvertebrate community composition and the influence of pesticides on the same.

The snails and insects collected from the area for the acute toxicity tests may have physiological differences to their temperate relatives they were compared to due to the lack of seasons which allows for year-round growth and activity in the macroinvertebrates. The tropical conditions also allow for year-round crop growth which means that agrochemical compounds tend to be used throughout the year. This may also mean that the insects from the region are exposed to the compounds more regularly and thus there is a higher chance of resistance in these insect. Nevertheless, as our acute toxicity tests showed that for a majority of insects, the differences between the taxa tested and their European relatives were negligible. This was true for all the taxa aside from coenagrionidae and corixidae, which had to be adjusted for the calibration. However, it must be noted that the SPEARpesticide analysis utilizes at least thirty taxa when analysing the macroinvertebrate community composition (Liess & Von Der Ohe, 2005). Thus, to accurately assess the impact of pesticide, acute toxicity tests of an additional nine taxa is required.

The findings here serve as a foundation of information of ecotoxicology information on the macroinvertebrates of western Kenya, especially centred around the *Schistosoma* parasite's host and their trophic interactive counterparts (competitors or predators). As such, only a maximum of 21 macro-invertebrates were tested for only two compounds, from only two categories of pesticides - a neonicotinoid and an organophosphate. The acute toxicity could still not evaluate the LC_{50} of all 21 species as the tolerance of the *Schistosoma* host snails – *Biomphalaria pfeifferi* and *Bulinus africanus*, was so high it could not be calculated as it surpassed the solubility of the test compounds. An explanation for this may also be that the pesticides chosen for experiment were those that were detected in snail tissues most frequently. This was due to the fact that pesticide data from organic material was the first to be made available, and thus experiments began using this preliminary data limited to a small number of compounds (Kandie *et al.*, 2020a). As such, the experiments may have inadvertently been carried out on compounds that have no effect, which would be the reason the snails were so commonly found to contain them, while the compounds that directly affect the snails would lead to their demise in the wild, and would be under-represented in our samples. Nevertheless, to build on the foundation laid out above, more classes of compounds need investigating, as well as on a higher number of species to get the whole picture. Furthermore, as our investigations were limited to pesticides, the effect of other compounds

such as herbicides, which affect the snail ecosystem by eliminating the snails' food source which is the aquatic vegetation and detritus (Abdel-Ghaffar *et al.*, 2016). Other compounds not tested but are of potential interest are fertilizers, which have been shown to increase snail host density by increasing food sources (Halstead *et al.*, 2018).

Chapter 4 investigated the effects of pesticides on the parasite itself. The study initially aimed at conducting experiments on the two *Schistosoma* species widespread in Kenya and the wider Africa, *Schistosoma mansoni* and *Schistosoma haematobium*. However, logistical difficulties brought by the COVID-19 pandemic restricted movement across counties, which limited access to areas where Bulinus snails were prominent and urinary schistosomiasis infections could be found. Thus, all experiments conducted in Chapter 4 were limited to the intestinal schistosomes - *Schistosoma mansoni*. I also observed that *Schistosoma mansoni* could be variable in its infectivity within our study area, such that miracidia from Mbita, Homa Bay County, were less infective than those from Katito, Kisumu County (*unpublished*). This variability within such close localities raise concerns about extrapolating the results continentally, as more differences may be present within *Schistosoma mansoni* found across the continent, not just in infectivity but perhaps pesticide tolerance as well. The probability in differences being observed increases when considering other related *Schistosoma* species that occur across the globe. Thus, further research must be conducted on the various *Schistosoma* parasites of interest as well as the effects of other agrochemical compounds as well.

Chapter 5 deals with calibrating the SPEARpesticide bioindicator from the standards and sensitivities of temperate insects from Germany to those of the tropical insects found in Kenya. While the major limitation was listed above, which was the lack of data on 9 taxa to confirm the sensitivity data aligned with that of Europe. However, the SPEARpesticide bioindicator is itself already limited in that, "because species-level data is aggregated according to sensitivity and life-cycle traits related to recovery, the effect of a pesticide cannot be assigned to any particular taxon" (Liess & Von Der Ohe, 2005).

6.3 Conclusions

i. The study determined that the distribution of schistosome-host snails occur across all the different littoral habitats found in the surrounding Lake Victoria area.
ii. The study also determined that the distribution of the snails is influenced by the presence of competitors and pesticide pollution such that increased pesticide pollution reduces

abundance of competitors of the snail thereby increasing snail dominance in polluted streams.

iii. The study determined that the infectivity of *Schistosoma* on snails does not reduce in presence of pesticides at environmentally relevant concentrations. The miracidia are hindered in their host seeking in pesticide polluted waters but only at concentrations up to 60 times what is found in the field. Sporocyst development within the snail is unaffected.

6.4 Recommendations

i. Implement disease surveillance: monitor the presence of host snails (infected and non-infected) in where waterbodies are utilised by locals for domestic purposes or could be in the future.

ii. Utilise the SPEARpesticide bio-indicator among other tools to help predict the spread of host snails due to pesticide pollution.

iii. Reduce agrochemical input into water bodies through the use of buffer strips.

iv. Reduce overall agrochemical use by turning to alternatives and aiming to develop alternatives.

v. Remove current pesticidal compounds from the environment through pesticide remediation plants.

vi. Continue research on the ecology of the host snails to determine the water parameters that dictate their presence. This study found several factors to be correlated with and against snails and further research could allow for predictions into expansion of range of the host snails and thus expansion of range of disease transmission risk.

vii. The study located several sites where infected snails could be present. Not only should research focus at these localities but health officials can also investigate these sites and use targeted intervention approaches to combat disease transmission in the area.

viii. Continue research on agrochemical effects on the ecosystem, as the compounds chosen based on preliminary work and therefore there leaves a lot of scope for research.

ix. Continue research on $SPEAR_{pesticide}$ application in Kenya and East Africa in general. This study began laying the foundations for its use by confirming that majority of the tested organisms from the study region have similar sensitivities to those in the databases which extract trait information from European taxa. This study tested 21 taxa, whereas for more precise resolution, 30 or more taxa should be tested. Thus, to continue building the accuracy and analytical strength of the African $SPEAR_{pesticide}$ application, more acute toxicity tests need to be conducted to improve the INDICATE program's pesticide concentration estimation power.

x. Research on the effect of multiple stressors on the snail and the macroinvertebrate community as heat usually exacerbates the effects of pesticides and thus, with climate change, the effects in the field may be worse than described in this study.

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APPENDICES

Appendix A: Supplementary Information for chapter 3

Appendices A.1: Table 3.S1: Classification of macroinvertebrate taxa in relation to the host snails of schistosomiasis.

Order	Family	Feeding type	Relation to snails
Ephemeroptera	Baetidae	Grazer	Competitor
Ephemeroptera	Caenidae	Grazer	Competitor
Ephemeroptera	Ephemeridae	Grazer	Competitor
Ephemeroptera	Heptageniidae	Grazer	Competitor
Ephemeroptera	Leptophlebiidae	Grazer	Competitor
Ephemeroptera	Oligoneuridae	Grazer	Competitor
Ephemeroptera	Polymitarcyidae	Grazer	Competitor
Ephemeroptera	Prosopistomatidae	Grazer	Competitor
Ephemeroptera	Teloganodidae	Grazer	Competitor
Plecoptera	Perlidae	Predator	Predator
Trichoptera	Ecnomidae	Predator, Grazer	Predator, Competitor
Trichoptera	Hydropsychidae	Filterer	Other
Trichoptera	Pisuliidae	Grazer	Competitor
Trichoptera	Polycentropodidae	Filterer	Other
Zygoptera	Chlorocyphidae	Predator	Predator
Zygoptera	Chlorolestidae	Predator	Predator
Zygoptera	Coenagriidae	Predator	Predator
Zygoptera	Lestidae	Predator	Predator

Anisoptera	Aeshnidae	Predator	Predator
Anisoptera	Corduliidae	Predator	Predator
Anisoptera	Gomphidae	Predator	Predator
Anisoptera	Libellulidae	Predator	Predator
Heteroptera	Belostomatidae	Predator	Predator
Heteroptera	Corixidae	Predator, Grazer	Predator, Competitor
Heteroptera	Gerridae	Predator	Predator
Heteroptera	Hydrometridae	Predator	Predator
Heteroptera	Naucoridae	Predator	Predator
Heteroptera	Nepidae	Predator	Predator
Heteroptera	Notonectidae	Predator	Predator
Heteroptera	Veliidae	Predator	Predator
Coleoptera	Dytiscidae	Predator	Predator
Coleoptera	Elmidae / Dryopidae	Grazer	Competitor
Coleoptera	Gyrinidae	Predator	Predator
Coleoptera	Haliplidae	Predator, Plant sucker	Predator, Other
Coleoptera	Hydrophilidae	Predator	Predator
Coleoptera	Noteridae	Predator	Predator
Coleoptera	Scirtidae	Predator	Predator
Diptera	Chironomidae	Filterer, Grazer	Other
Diptera	Culicidae	Filterer, Grazer	Competitor, Other
Diptera	Muscidae	Predator	Predator

Diptera	Simulidae	Filterer	Other
Diptera	Tipulidae	Shredder	Other
Lepidoptera	Pyralidae	Herbivore	Competitor
Crustacea	Atyidae	Grazer, Filterer	Competitor
Gastropoda	Ampulariidae	Grazer, Herbivore, Predator	Snail
Gastropoda	Ancylidae	Grazer	Snail
Gastropoda	Hydrobiidae	Grazer	Snail
Gastropoda	Lymnaeidae	Grazer, Herbivore	Snail
Gastropoda	Physidae	Grazer	Snail
Gastropoda	Planorbidae	Grazer, Herbivore	Snail
Gastropoda	Thiaridae	Grazer	Snail
Gastropoda	Viviparidae	Grazer	Snail
Annelida	Hirudinea	Predator	Predator
Annelida	Oligochaeta	Detritivore	Other

Field Sampling Water Datasheet

Name:

Date of sampling:

Professional Status:

Sampling Sites ID						

Odour

H_2S						
soap musty						
earthy sewage						
Other						

Colour

brown						
grey green						
white						
colourless Other:						

Floating matter

algal bloom						

Appendix A.2: Figure 3.S1. Site physicochemical and ecological characteristics data sheet.

SENTINEL-icipeTOC AG-SOP-1		Taxon		SW	DP	GSM	TOT	Taxon		SW	DP	GSM	TOT	Taxon		SW	DP	GSM	TOT
DATE:	**TIME:**	**PORIFERA**	5					**HEMIPTERA**						**DIPTERA**					
		COELENTERATA	1					Belostomatidae*	3					Athericidae	10				
SITE CODE:		**TURBELLARIA**	3					Corixidae*	3					Blepharoceridae	15				
Longitude		**ANNELIDA**						Gerridae*	5					Ceratopogonidae	5				
Lattitude		Oligochaeta	1					Hydrometridae*	6					Chironomidae	2				
Altitude		Leeches	3					Naucoridae*	7					Culicidae*	1				
WEATHER:		**CRUSTACEA**						Nepidae*	3					Dixidae*	10				
		Amphipoda	13					Notonectidae*	3					Empididae	6				
HABITAT TYPE:		Potamonautidae*	3					Pleidae*	4					Ephydridae	3				
Permanent river Main	Rice field	Atyidae	8					Veliidae/M...veliidae*	5					Muscidae	1				
Minor tributary	Channel	Palaemonidae	10					**MEGALOPTERA**						Psychodidae	1				
Headwater stream	Dam	**HYDRACARINA**	8					Corydalidae	8					Simuliidae	5				
Seasonal river	Puddle/pool/furrow	**PLECOPTERA**						Sialidae	6					Syrphidae*	1				
LANDCOVER:		Notonemouridae	14					**TRICHOPTERA**						Tabanidae	5				
Natural	Urban	Perlidae	12					Dipseudopsidae	10					Tipulidae	5				
Agricultural	Industrial	**EPHEMEROPTERA**						Ecnomidae	8					**GASTROPODA**					
Semi-urban		Baetidae 1sp	4					Hydropsychidae 1 sp	4					Ancylidae	6				
LAND USE:		Baetidae 2 sp	6					Hydropsychidae 2 sp	6					Bulininae*	3				
Forest:	Natural dryland forest	Baetidae > 2 sp	12					Hydropsychidae > 2 sp	12					Hydrobiidae*	3				
	Natural riverine forest	Caenidae	6					Philopotamidae	10					Lymnaeidae*	3				
	Reforestation	Ephemeridae	15					Polycentropodidae	12					Physidae*	3				
Grassland:	Shrubland	Heptageniidae	13					Psychomyiidae/Xiphocent	8					Planorbinae*	3				
	Savanna	Leptophlebiidae	9					**Cased caddis:**						Thiaridae*	3				
Agriculture:	Commecrial	Oligoneuridae	15					Barbarochthonidae SWC	13					Viviparidae* ST	5				
	Subsistance	Polymitarcyidae	10					Calamoceratidae ST	11					**PELECYPODA**					
	Irrigation Scheme	Prosopistomatidae	15					Glossosomatidae SWC	11					Corbiculidae	5				
	Agroforestry	Teloganodidae SWC	12					Hydroptilidae	6					Sphaeriidae	3				
Crop type:	Tea	Tricorythidae	9					Hydrosalpingidae SWC	15					Unionidae	6				
	Sugar	**ODONATA**						Lepidostomatidae	10					**SASS Score**					
Other:	Rice	Calopterygidae ST,T	10					Leptoceridae	6					**No. of Taxa**					
	Maize	Chlorocyphidae	10					Petrothrincidae SWC	11					**ASPT**					
HUMAN ACTIVITY:		Chlorolestidae	8					Pisuliidae	10					**Other biota including juveniles:**					
Bathing/washing	Animal feeding	Coenagrionidae	4					Sericostomatidae SWC	13										
Water collection	Other:	Lestidae	8					**COLEOPTERA**											
Sand harvesting		Platycnemidae	10					Dytiscidae*	5					**Comments:**					
AQUATIC HABITAT VEGETATION:		Protoneuridae	8					Elmidae/Dryopidae*	8										
Emerging	Dominant sp.:	Aeshnidae	8					Gyrinidae*	5										
Floating	Dominant sp.:	Corduliidae	8					Haliplidae*	5										
Submerged	Dominant sp.:	Gomphidae	6					Helodidae	12										
Hanging	Dominant sp.:	Libellulidae	4					Hydraenidae*	8										
%SHADE:	**%DETRITUS:**	**LEPIDOPTERA**						Hydrophilidae*	5										
NOTES:		Pyralidae	12					Limnichidae	10										
								Psephenidae	10										

Appendices A.3: Figure 3.S2. Macroinvertebrate sampling data sheet adapted from SASS5 (Dickens and Graham, 2002)**.**

Appendix B: Supplementary Information for chapter 4

Appendix B.1: Table 4.S1: Results of the miracidia host seeking assay in raw format.

Pesticide	Treatment	Concentration	Infected	Not infected	Percentage infected
Control	Control	0	3	9	25.86
Control	Control	0	2	10	
Control	Control	0	5	8	
Control	Control	0	5	7	
Control	Control	0	4	8	
Control	Control	0	0	13	
Imidacloprid	Low	4.88	4	8	44.44
Imidacloprid	Low	4.88	6	6	
Imidacloprid	Low	4.88	6	6	
Imidacloprid	High	48.8	0	12	2.78
Imidacloprid	High	48.8	0	12	
Imidacloprid	High	48.8	1	11	
Diazinon	Low	1.05	1	11	11.11
Diazinon	Low	1.05	0	12	
Diazinon	Low	1.05	3	9	
Diazinon	High	10.5	0	12	2.78
Diazinon	High	10.5	0	12	
Diazinon	High	10.5	1	11	

Appendix B.2: Table 4.S2: Results of the sporocyst development assay in raw format.

Pesticide	Treatment	Concentration	Shedding	Not shedding	Percentage shedding
Control	Control	0	1	7	21.03
Control	Control	0	1	5	
Control	Control	0	1	5	
Control	Control	0	1	6	
Control	Control	0	2	5	
Control	Control	0	3	5	
Imidacloprid	Low	4.88	3	5	23.61
Imidacloprid	Low	4.88	0	8	
Imidacloprid	Low	4.88	2	4	
Imidacloprid	High	48.8	2	2	33.33
Imidacloprid	High	48.8	0	3	
Imidacloprid	High	48.8	3	3	
Diazinon	Low	1.05	0	3	26.67
Diazinon	Low	1.05	4	1	
Diazinon	Low	1.05	0	4	
Diazinon	High	10.5	2	2	27.78
Diazinon	High	10.5	2	4	
Diazinon	High	10.5	0	7	

Appendix B.3: Consent forms

Kenya Medical Research Institute (KEMRI), Kenya.

INFORMED CONSENT EXPLANATION

PROJECT TITLE: Effect of agrochemical pollutants on the infectivity and maturation of *Schistosoma haematobium* and *Schistosoma mansoni* in the Lake Victoria basin

Pis: Eric Lelo Agola, Ulrike Fillinger, Faith Kandie and Akbar Ganatra

INTRODUCTION AND PARTICIPATION INFORMATION: The Kenya Medical Research Institute (KEMRI) is conducting a study on schistosomiasis (commonly known as bilharzia) in Homabay and Kisumu Counties Kenya. Bilharzia afflicts millions of people throughout the world, and it is caused by parasitic worms which are transmitted by snails that live in water. The investigators are requesting your participation in this study. Participation is entirely voluntary, and you may be included as a participant in the study if you or your parent/guardian gives consent. Even when consent has been given for participation, you may withdraw if you so wish at any time, without penalty or loss of benefit to which you are otherwise entitled. When you have read this explanation, please feel free to ask questions or to seek clarification on any issues related to this study or your participation in it, both before consenting and at any time thereafter. The study has been approved by the KEMRI Scientific Steering Committee (SSC) and the KEMRI/National Ethical Review Committee.

PURPOSE OF THE STUDY: The purpose of this study is to determine the effect of different agrochemical pollutants on Schistosomiasis transmission by studying their effect on different *Schistosoma*-life stages. This will determine the survival and infectivity of the parasite in three different aquatic life stages and ultimately determine whether transmission is aided or reduced.

PROCEDURES TO BE USED: I will recruit you from your school to include you in the study only if you agree to participate and your parents sign a consent form to allow you to participate. If they agree to let you participate in this study, you will be asked to answer a few questions

concerning symptoms associated with schistosomiasis. I shall then require you to give us a stool sample, which I shall examine for eggs of the bilharzia parasites, as well as other intestinal helminths. I may require you to give additional stool samples.

MAINTENANCE OF CONFIDENTIALITY: Your identity and test results will remain confidential. As a study participant, you will be assigned a number, and you or results of tests done on samples taken from you will be referred to by this number in all correspondence or publications arising from this study. All information and medical records will be confidential.

BENEFITS: If you are diagnosed with schistosomiasis you will be treated with praziquantel (40mg/kg body wt) after the initial or final stool screening. If you have other intestinal helminthes (e.g. *Ascaris, Trichuris*), you will be treated for these infections as well, regardless of your schistosomiasis infection status. You will also benefit by access to occasional examination by the collaborating physician, and prescription of drugs for other ailments other than those mentioned above. Purchasing of any other drugs prescribed by the physician, other than praziquantel, and anti-helmintics, will however be your responsibility.

RISKS, HAZARDS AND DISCOMFORTS ASSOCIATED WITH THE PROCEDURES: Collection of stool samples from you is not considered hazardous. The medication you will receive for treatment of bilharzia or other parasitic infections diagnosed is considered safe with minimal side effects.

INFORMED CONSENT AGREEMENT FOR CHILDREN

I, Mr./Mrs/Miss , being a person aged 18 years and over, and being the parent/guardian of:

Msr/Miss (Child's name) Age

Name of School House Number do hereby give permission to Prof/Dr./Mr./Mrs/Miss

to include her/him in the proposed study titled **"Effect of agrochemical pollutants on the infectivity and maturation of *Schistosoma haematobium* and *Schistosoma mansoni* in the Lake Victoria basin"** which has already been explained to me to my satisfaction. I have been informed about the procedures to be used on my child and the hazards, risks or benefits associated with these to Mr/Miss have been explained to me clearly. I accept the investigators to take stool samples from the child, and I accept the child to receive the medication to be provided through this study. I understand that I may withdraw the child from participating in this investigational study any time I wish, without penalty or loss of benefits he/she may be entitled to. All the issues concerning this study have been explained to me in the language, which I speak fluently and understand clearly.

Signature (or Thumb Print) of Parent/Guardian

Date

Name of the Person Obtaining Consent and Signature

Name and Signature of Witness

TREATMENT CONSENT:

If your child has the infection, he/she can be treated for it by trained people from KEMRI. If he/she has other infections caused by worms, he/she can be treated for those also. The treatments are free. Is it okay for your child to receive treatment if he/she has a worm infection?

Yes No

Signature (or Thumb Print of Parent/Guardian

ASSENT FOR CHILDREN:

You are being asked to provide stool samples so that I can check to see if you have any worms living in your body. If I find worms, you will get some medication to make you better. You don't have to do this if you don't want but there is no danger if you do. It might help you. Do you agree to give us stool samples to check for the worms?

Yes No

Name of the Child

Name of the Person Obtaining Consent and Signature

Name and Signature of the Witness

Kambi mar timo nonro mar thieth mar Kenya (KEMRI), Kenya.

WECHE MALERO CHIWO THUOLO

WII NONRO: Lokrwok mar kemikol mag pur mochido kwom nyalo mar iko tuo kod tegno mar njokni milwongo ni "*Schistosoma haematobium*" kod "*Schistosoma mansoni*" e alwora mar ataro mar viktoria

Joma Ochung' ni nonro en: Eric Lelo Agola, Ulrike Fillinger, Faith Kandie kod Akbar Ganatra

CHAKRUOK KOD WECHE MOTENORE GI TIO KANYAKLA: Kambi mar timo nonro mar thieth mar Kenya miluongo ni (KEMRI) timo nonro ewi tuo milwongo gi tho ngere ni schistosomiasis (ma bende ong'ere kaka bilharzia) e aluora mar Machakos-

Kitui piny Kenya. Bilharzia sando tara gi gana mar dhano e piny magima. Ikele gi njokni ma be ilande gi tung' kamnio modak ei pi. Jo nonro kwayo Bedoni kanyakla ei nonro ni. Bedo kanyakla en kwom chiurwok kendo inyalo keti mondo ibed kanyakla ei nonro ni ka in kata janyuolni ochiwo thuolo. Kata ka thuolo osechiu kwom bedo kanyakla, inyalo wuok kinde moramora ka idwaro , ka ok ogoi fain, ka onge kum kata lalo ber mosemiyi. Ka isesomo weche moler kaye, bed thuolo kwom penjo penjo kata manyo ler kwom wach moramora motenore gi nonro ni kata bedo ni kanyakla, duto ka pok ichiwo thuolo kendo e kinde moramora bang'e. Nonro ni opwodhi gi komiti matayo nonro ma en KEMRI kod KEMRI/komiti mang'iyo ratich dhano mar piny e nonro.

GIMA OMIYO ITIMO NONRO: Gima omiyo itimo nonro ni en fwenyo lokrwok mar kemikol mag pur mochido mopogore kwom pogo tuo milwongo ni "Schistosomiasis" kwom somo lokrwok gi e tieng' dongo mopogore mar kudini milwongo ni "*Schistosoma".* Maa biro fwenyo ngima kata bedo gi nyalo mar tuo dongo kwom njokni e tieng' adek mar ngima gi ei pi. Kendo kwom kinde moko ng'iyo ka tuo nyalo landore kata dok piny.

Ratiro mibiro tiyo go: Wabiro rwako uu koya kwom sikunduu mondo omi waketu e nonro ka iyie bedo kanyakla kendo ka janywolni ochwo koke e barua mar yieni mondo ibed kanyakla kod nonro. Ka giyieni bedo achiel e nonro ni, ibiro kwayi mondo idwok penjo matin

motenore gi ranyisi moluore gi two miluongo ni "schistosomiasis" ma en two mikelo gi njokni mag ndokchin. Wabiro dwaro mondo imiwa lachni, ma wabiro timo ni nonro mar ng'iyo tong' njokni makelo bilharzia, kata ng'iyo njokni moko mag dhokchin. Wanyalo dwaro mondo imedwa lach moko.

Rito maling'ling': Ng'eruok mari kata duoko mar pim biro bedo maling'ling. Kaka jalno matiyo kanyakla gi nonro ni, ibiro miyi namba kendo in kata dwoko mag pim motim koya kwom gigo mokau kwomi, ibiro ng'igi kokalo kwom namba momiyi no bende endiko moramora kokalo kwom nonro ni ibiro ti gi namba no. Weche duto te kod gige ni mag thieth biro bedo maling'ling'.

Ber: Ka iyudori gi two miluongo ni schistosomiasis (mikelo gi njokni) ibiro thiedhi gi yath miluongo ni praziquantel (40mg/kg body wt) bang' pimo mokuongo gi mogik mar lachni. To ka in gi njokni mag dhokchin (kaka *Ascaris, Trichuris*), ibiro thiedhi kuom ma be, kok odeo chal mari mar two mar schistosomiasis. Bende ibiro yudo ber mar

yudo pimo mar ngimani mar kinde ka kinde mitimo gi Laktar matiyo kanyakla gi nonro ni bende ibiro lerni yiedhe mag tuoche moko mopogore gi two ma wawuoye malo kanyo. Nyiewo yiedhe moko molerni gi laktar mopogore gi praziquantel, kata yiethe manego njokni, biro bedo ewiyi.

YUDO KATA LALO, RACH KOD LIT MATIN MOTENORE GI RATIRO:

Kawo lach koya kwomi ok ng'i ka gima rach. Thieth mibiro yudo kwom thiedho bilharzia kata tuo motenore njokni mamoko mopim ing'iyo ka gima kare mani gi rem matin.

CHIWO THUOLO MAKARE GI WINJRUOK KUOM NITHINDO

An, Mr./Mrs/Miss , bedo ng'ano mani gi higni 18 gi malo, kendo bedo janyuol kata japid mar:

Mrs. /Miss (Nying nathi) Higa

Nying Skul Namba mar ot chiwo thuolo ni Prof/Dr./Mr./Mrs/Miss

Mondo okete e nonro mokwa ma wiye malo macho niya "Effect of agrochemical pollutants on the infectivity and maturation of *Schistosoma haematobium* and *Schistosoma mansoni* e aluora mar ataro mar Victoria" ma oselerna eyo makare ma ayiego. Osenyisa ratiro mibiro luo kuom nathina gi rach kata ber motenore gi ma kuom Mr/Miss oselerna eyo maler. Ayie jononro mondo okau rapim matin mar chieth koya kuom nathi kendo ayie nathi mondo oyud thieth michiwo kokalo kuom nonro ni. An gi ng'eyo ni anyalo golo nathi kuom bedo kanyakla e nonro ni e kinde mora mora ma adwaro ma ok abedo gi fain kata lalo ber manyalo bedo ni omiye. Weche duto te motenore gi nonro ni oselerna gi dhok (dholuo) ma awacho maler kendo awinjo maber.

Sei (kata Ranyisi mar kogno) mar Janyuol/Japid

Tarik

Nying ng'ano mayudo thuolo gi sei

Nying gi sei mar Janeno

CHIWO THUOLO KWOM THIETH:

Ka nyathini ni gi tuo, inyalo thiedhe gi jogo molony moya KEMRI. To ka en gi tuoche moko mokel gi njokni, inyalo thiedhe kwom mago be. Thieth en nono. Be ber ka nyathini yudo thieth ka oyudore gi tuo mokel gi njokni?

Ayie Adagi

sei (kose Ranyisi mar kogno mar Janyuol/Japid

CHIWO THUOLO MAR NYITHINDO

Ikwayi mondo ichiu lachni mondo omi wang'ii ka in gi njokni modak e dendi. Ka wayudo njokni, ibiro yudo thieth mondo omiyi ibed maber. Ok'ochuno mondo itim ma aka ok idwar to onge rach ka itimo. Nyalo konyi. Be iyie miwa lachni mondo wang'ii njokni?

Ayie Adagi

Nying Nyathi

Nying ng'ano machiwo thuolo gi sain mare

Nying gi sain mar Janeno

Appendix C.1: Research License (NACOSTI) 2022

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 550858

Date of Issue: 25/February/2022

RESEARCH LICENSE

This is to Certify that Mr.. Akbar Abdulaziz Ganatra of Egerton University, has been licensed to conduct research in Homabay on the topic: ecotoxicological investigation into the effect of pesticide pollution on schistosomiasis transmission for the period ending : 25/February/2023.

License No: NACOSTI/P/22/15921

550858

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix C.2: Research Licence (NACOSTI) 2021

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 747464

Date of Issue: 15/February/2021

RESEARCH LICENSE

This is to Certify that Mr.. Akbar Abdulaziz Ganatra of Egerton University, has been licensed to conduct research in Homabay on the topic: Ecotoxilogical investigation into the effect of freshwater pollution on schistosomiasis for the period ending : 15/February/2022.

License No: NACOSTI/P/21/8925

747464

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix C.3: Research Licence (NACOSTI) 2020

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 596190

Date of Issue: 20/January/2020

RESEARCH LICENSE

This is to Certify that Mr.. Akbar Ganatra of Egerton University, has been licensed to conduct research in Homabay on the topic: Ecotoxilogical investigation of the effects of agrochemicals on transmission of schistosomiasis in western Kenya for the period ending : 20/January/2021.

License No: NACOSTI/P/20/2217

596190

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix D.1: Ethics Approval 2022

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) 2722541, 2713349, 0722-205901, 0733-400003, Fax: (254) (020) 2720030
Email: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **April 04, 2022**

TO: **DR. ERIC LELO,**
PRINCIPAL INVESTIGATOR.

THROUGH: **THE DEPUTY DIRECTOR, CBRD,**
NAIROBI.

Dear Sir,

RE: **PROTOCOL NO. SERU 3836 *(RESUBMITTED REQUEST FOR ANNUAL RENEWAL)*: EFFECTS OF IMIDACLOPRID AND DIAZINON ON THE INFECTIVITY AND MATURATION OF *SCHISTOSOMA HAEMATOBIUM* AND *SCHISTOSOMA MANSONI* IN THE LAKE VICTORIA BASIN.**

Reference is made to your letter dated April 01, 2022. The KEMRI Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised study documents on April 01, 2022.

This is to inform you that the Expedited Review Team of the SERU determined that the issues it raised on the letter dated **March 11, 2022,** have been adequately addressed.

Consequently, the study is **granted approval for** continuation effective **April 04, 2022,** through to **April 3, 2023**. Please note that authorization to conduct this study will automatically expire on **April 3, 2023**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the SERU by **February 20, 2023**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise them when the study is completed or discontinued.

Yours faithfully,

PROF. CHARLES OBONYO,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

Appendix D.2: Ethics Approval 2021

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) 2722541, 2713349, 0722-205901,0733-400003, Fax: (254) (020) 2720030
Email: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **March 31, 2021**

TO: **DR. ERIC LELO**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DEPUTY DIRECTOR, CBRD**
NAIROBI

Dear Sir,

RE: **SERU PROTOCOL NO. 3836 (*REQUEST FOR ANNUAL RENEWAL*): EFFECTS OF IMIDACLOPRID AND DIAZINONON THE INFECTIVITY AND MATURATION OF *SCHISTOSOMA HAEMATOBIUM* AND *SCHISTOSOMA MANSONI* IN THE LAKE VICTORIA BASIN.**

Thank you for the continuing review report for the period **April 11, 2019 to February 27, 2021.**

This is to inform you that the Expedited Review Team of the KEMRI Scientific and Ethics Review Unit (SERU) was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted **approval** for continuation.

This approval is valid from **April 11, 2021** through to **April 10, 2022**. Please note that authorization to conduct this study will automatically expire on **April 10, 2022**. If you plan to continue with data collection or analysis beyond this date please apply for continuing approval to the SERU by **February 27, 2022.**

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SERU for review prior to initiation.

Yours faithfully,

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

Appendix D.3: Ethics Approval 2020

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel: (254) 2722541, 2713349, 0722-205901,0733-400003, Fax: (254) (020) 2720030
Email: director@kemri.org, info@kemri.org, Website. www.kemri.org

KEMRI/RES/7/3/1 **March 25, 2020**

TO: **DR. ERIC LELO,**
PRINCIPAL INVESTIGATOR

THROUGH: **THE DIRECTOR, CBRD**
NAIROBI

Dear Sir,

RE: **KEMRI/SERU/CBRD/194/3836 *(REQUEST FOR ANNUAL RENEWAL)*: EFFECT OF IMIDACLOPRID AND DIAZINON ON THE INFECTIVITY AND MATURATION OF *SCHISTOSOMA HAEMATOBIUM* AND *SCHISTOSOMA MANSONI* IN THE LAKE VICTORIA BASIN.**

Thank you for the continuing review report for the period **April 11, 2019** to **February 25, 2020.**

This is to inform you that the Expedited Review Team of the KEMRI Scientific and Ethics Review Unit (SERU) was of the informed opinion that the progress made during the reported period is satisfactory. The study has therefore been granted **approval.**

This approval is valid from **April 11, 2020** for a period of **one (1) year.** Please note that authorization to conduct this study will automatically expire on **April 10, 2021.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval by **February 27, 2021.**

You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to the SERU for review prior to initiation. You may continue with the study.

Yours faithfully,

ENOCK KEBENEI,
THE ACTING HEAD,
KEMRI SCIENTIFIC AND ETHICS REVIEW UNIT.

Appendix D.4: Ethics Approval 2019

Appendix E: Approval for using school children by ministry of education

MINISTRY OF EDUCATION

STATE DEPARTMENT FOR EARLY LEARNING & BASIC EDUCATION

Telegrams: "SCHOOLING" Homa Bay
Telephone +
When replying please quote
cdehomabay@gmail.com

COUNTY DIRECTOR OF EDUCATION
HOMA BAY COUNTY
P.O BOX 710
HOMA BAY
DATE: 17TH FEBRUARY, 2020

REF: MOEST/CDE/HBC/ADM/11/VOL. II/47

Mr. Akbar Ganatra
Egerton University

RE: RESEARCH AUTHORIZATION.

Following your application for authority to carry out research on ***"Ecotoxilogical investigation of the effects of agrochemicals on transmission of schistosomiasis in western Kenya"*** I am pleased to inform you that you have been authorized to undertake research in Homa Bay County for the period ending **20th January, 2021.**

Kindly note that ,as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit a copy of the final research report to the County Director of Education Office after completion both the soft copy and hard copy.

Thank you in advance.

COUNTY DIRECTOR OF EDUCATION
HOMA BAY COUNTY
P O BOX 710-40300, HOMA BAY
Email: cdehomabay@gmail.com

MR. SHEM OMBONYO
FOR: COUNTY DIRECTOR OF EDUCATION
Cc.

1. **County Commissioner**
   Homa Bay County.
2. **County Director Health**

Appendix F: Approval for using school children by ministry of health

MINISTRY OF HEALTH

Telegrams: "MOH" Homa Bay

MINISTRY OF HEALTH

Appendix G: Certificate of Reviewing

Appendix H.1: Publication abstract 1

www.nature.com/scientificreports

SCIENTIFIC REPORTS

nature research

Corrected: Author Correction

OPEN

Pesticide pollution in freshwater paves the way for schistosomiasis transmission

Jeremias M. Becker[1,2,9], Akbar A. Ganatra[3,4,9*], Faith Kandie[2,3], Lina Mühlbauer[1,5], Jörg Ahlheim[1], Werner Brack[1,2], Baldwyn Torto[3], Eric L. Agola[6,7], Francis McOdimba[3,4], Henner Hollert[2,8], Ulrike Fillinger[3*] & Matthias Liess[1,2*]

Schistosomiasis is a severe neglected tropical disease caused by trematodes and transmitted by freshwater snails. Snails are known to be highly tolerant to agricultural pesticides. However, little attention has been paid to the ecological consequences of pesticide pollution in areas endemic for schistosomiasis, where people live in close contact with non-sanitized freshwaters. In complementary laboratory and field studies on Kenyan inland areas along Lake Victoria, we show that pesticide pollution is a major driver in increasing the occurrence of host snails and thus the risk of schistosomiasis transmission. In the laboratory, snails showed higher insecticide tolerance to commonly found pesticides than associated invertebrates, in particular to the neonicotinoid Imidacloprid and the organophosphate Diazinon. In the field, we demonstrated at 48 sites that snails were present exclusively in habitats characterized by pesticide pollution and eutrophication. Our analysis revealed that insensitive snails dominated over their less tolerant competitors. The study shows for the first time that in the field, pesticide concentrations considered "safe" in environmental risk assessment have indirect effects on human health. Thus we conclude there is a need for rethinking the environmental risk of low pesticide concentrations and of integrating agricultural mitigation measures in the control of schistosomiasis.

Schistosomiasis, also called bilharzia, is among the tropical diseases with the highest impact on socio-economic development, only exceeded by malaria. Approximately 218 million people are infected worldwide[1]. Infection has been strongly associated with long-term disabilities[2]. The number of deaths due to schistosomiasis is poorly documented with estimates ranging between 11,700[3] to 280,000 each year[4] because of hidden pathologies such as liver and kidney failure[5]. Schistosomiasis is caused by flatworms of the genus *Schistosoma* which parasitize humans as their definitive host (supporting the adult life stage of the parasite). The intermediate hosts are freshwater snails of the family planorbidae which release infective larval stages (cercariae) into the water. Transmission occurs when humans are exposed to water containing infected host snails; direct infection from person to person is not possible[6]. People are infected during routine agricultural, domestic, occupational and recreational activities, which expose them to infested water. Over 80% of afflicted people live in sub-Saharan Africa[7], but the disease concerns public health in most (sub)tropical countries worldwide[7] and has recently established in Europe[8].

Control strategies against schistosomiasis focus on the treatment with praziquantel that kills the adult worms in the human host. However, even mass drug administration does not prevent re-infection in infested water, and schistosomiasis has been observed to rebound within short time[9]. For the sustainable control of schistosomiasis, it

[1]Helmholtz Centre for Environmental Research – UFZ, Department System-Ecotoxicology, Permoserstrasse 15, 04318, Leipzig, Germany. [2]RWTH Aachen University, Department of Ecosystem Analysis, Institute for Environmental Research, Worringerweg 1, 52074, Aachen, Germany. [3]International Centre of Insect Physiology and Ecology (icipe), Human Health department, P.O. Box 30772-00100, Nairobi, Kenya. [4]Egerton University, Biological sciences, P.O Box 536-20115, Njoro, Kenya. [5]Ruprecht-Karl-University of Heidelberg, Faculty of Biosciences, Im Neuenheimer Feld 234, 69120, Heidelberg, Germany. [6]Centre for Biotechnology Research and Development, Kenya Medical Research institute (KEMRI), P.O. Box 54840-00200, Nairobi, Kenya. [7]The Technical University of Kenya, P.O. Box 52428-00200, Nairobi, Kenya. [8]Department Evolutionary Ecology and Environmental Toxicology, Institute of Ecology, Evolution and Diversity, Faculty Biological Sciences, Goethe University Frankfurt, Frankfurt, 60438, Germany. [9]These authors contributed equally: Jeremias M. Becker and Akbar A. Ganatra. *email: aganatra@icipe.org; ufillinger@icipe.org; matthias.liess@ufz.de

Appendix H.2: Publication abstract 2

Ganatra et al. Environ Sci Eur (2021) 33:58
https://doi.org/10.1186/s12302-021-00497-9

Environmental Sciences Europe

RESEARCH Open Access

Calibration of the $SPEAR_{pesticides}$ bioindicator for cost-effective pesticide monitoring in East African streams

Akbar A. Ganatra[1,2], Faith Jebiwot Kandie[1,3,4,5], Ulrike Fillinger[1], Francis McOdimba[1,2], Baldwyn Torto[1], Werner Brack[3,5], Matthias Liess[6,7*], Henner Hollert[7] and Jeremias M. Becker[6,7]

Abstract

Background: Pesticides are washed from agricultural fields into adjacent streams, where even short-term exposure causes long-term ecological damage. Detecting pesticide pollution in streams thus requires the expensive monitoring of peak concentrations during run-off events. Alternatively, exposure and ecological effects can be assessed using the $SPEAR_{pesticides}$ bioindicator that quantifies pesticide-related changes in the macroinvertebrate community composition. $SPEAR_{pesticides}$ has been developed in Central Europe and validated in other parts of Europe, Australia and South America; here we investigated its performance in East African streams.

Results: With minimal adaptations of the $SPEAR_{pesticides}$ index, we successfully characterized pesticide pollution in 13 streams located in Western Kenya. The East African $SPEAR_{pesticides}$ index correlated well with the overall toxicity of 30 pesticides (maximum toxic unit = maximum environmental vs. median lethal concentration) measured in stream water ($R^2 = 0.53$). Similarly, the $SPEAR_{pesticides}$ index correlated with the risk of surface run-off from agricultural fields (as identified based on ground slope in the catchment area and the width of protective riparian strips, $R^2 = 0.45$). Unlike other bioindicators designed to indicate general water pollution, $SPEAR_{pesticides}$ was independent of organic pollution and highly specific to pesticides. In 23% of the streams, pesticides exceeded concentrations considered environmentally safe based on European first tiered risk assessment.

Conclusions: Increasing contamination was associated with considerable changes in the macroinvertebrate community composition. We conclude that pesticides need to be better regulated also in developing countries. $SPEAR_{pesticides}$ provides a straightforward and cost-efficient tool for the required monitoring of pesticide exposure in small to medium streams.

Keywords: Ecotoxicology, Bio-indicator, Pesticide pollution

Background

In 2020, the worldwide application of agricultural pesticides is expected to increase from 2 million tonnes to 3.5 million tonnes annually [1]. Pesticide pollution is considered one of the main drivers for the global decline in the abundance and diversity of insects, plants and birds [2–4]. There is increasing evidence that the pesticide-driven impairment of biocenoses also affects valuable ecosystem services ranging from pollination [5] to leaf-litter degradation [6] and to the biological control of agricultural pests [7, 8] and of pathogens in freshwater [9]. For the United States and the European Union, where pesticides are used on a large scale, extensive literature on exposure and effects in the environment is available from academic research and from regulatory risk assessment [1]. In developing countries,

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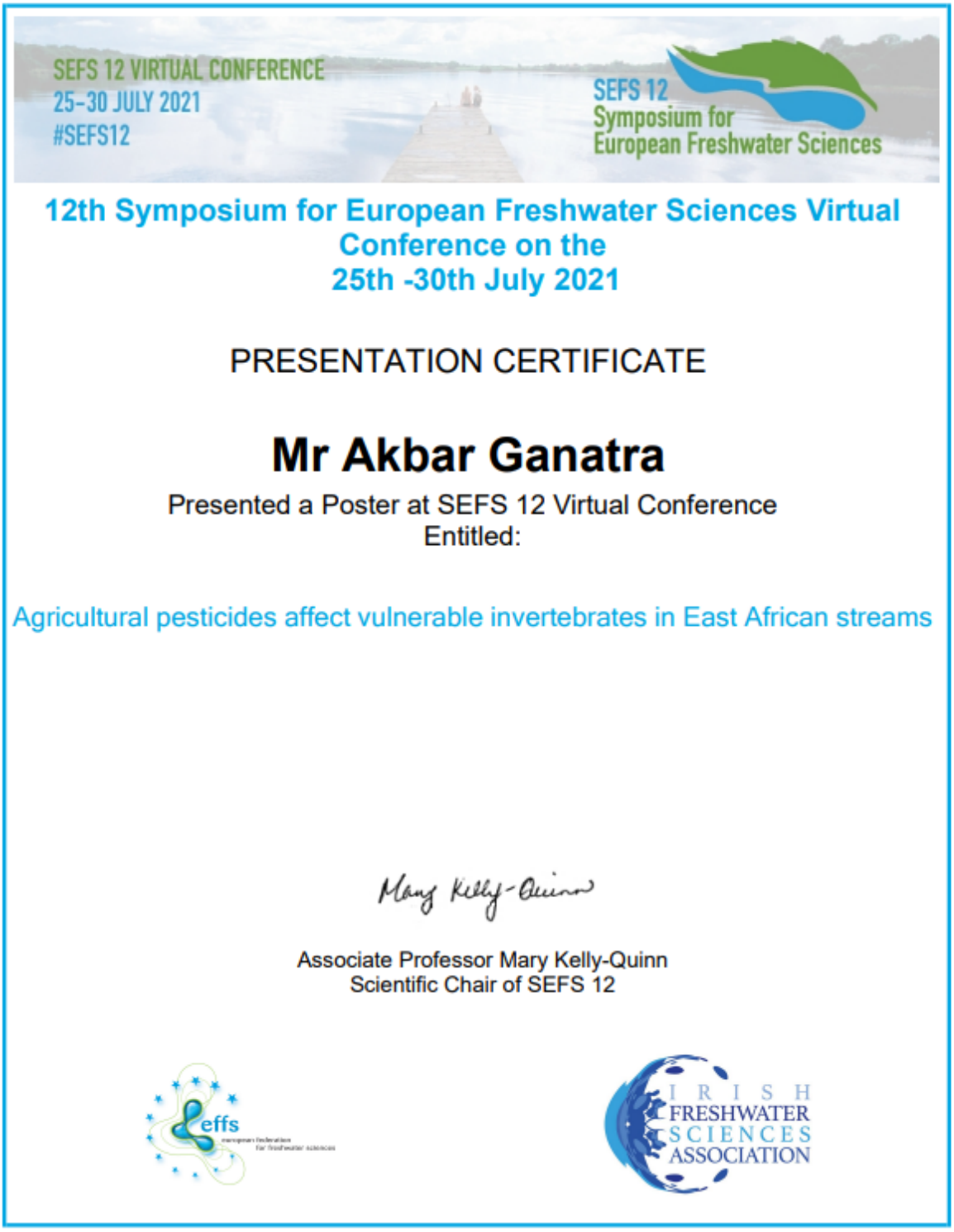
[6] Department of System-Ecotoxicology, Helmholtz Centre for Environmental Research GmbH – UFZ, 04318 Leipzig, Germany
Full list of author information is available at the end of the article

Appendix I: International conferences presented at:

Appendix I.1: SEFS conference certificate of participation



Appendix I.2: ECTMIH conference certificate of participation

11TH EUROPEAN CONGRESS ON TROPICAL MEDICINE AND INTERNATIONAL HEALTH

MR. AKBAR GANATRA
ICIPE
40305 MBITA
KENYA

CERTIFICATE OF ATTENDANCE

We hereby certify the participation of

AKBAR GANATRA

in the European Congress on Tropical Medicine and International Health on September 16 until September 20, 2019 in Liverpool, United Kingdom.

20/09/2019, Liverpool

Tamar Ghosh
CEO RSTMH and Director
ECTMIH 2019

Appendix I.3: NTD conference certificate of best poster

KENYA MEDICAL RESEARCH INSTITUTE
KEMRI

NEGLECTED TROPICAL DISEASES
End Suffering and Poverty, Let's Act Now

Certificate of Honour

This certificate is awarded to

AKBAR GANATRA

Honoured for the Best poster presentation during the 11th Annual MOH-KEMRI NTD Scientific Conference

Held on 6th-7th December 2017 at the Sarova Panafric Nairobi

Dr. Sultani H. Matendechero
Chair- Organizing Committee

Dr. Maurice Odiere
Co– Chair organizing Committee

Appendix J: Extracurricular activities

- Paper review – Environmental Advances ELSEVIER
- *Icipe* Scholar's Association (IScA) Mbita student representative 2019-2020
- Pesticide and Poisons Control Board Public Forum *icipe* Representative 28th September 2020
- Mbita Science Club Chairperson 2018-2019
- Busia OpenSpace Workshop Volunteer 20th – 22nd July 2021
- Introduction to Entomology lectures given:
    - Masinde Muliro University – 14th November 2019
    - Rangwe Mixed High school -12th July 2019
    - Icipe Mbita Malaria Day workshop 2021– select top students from 20 local schools
- Malaria Day 2018 planning committee
- icipe Scholar's Association (IScA) Team Building Getaway Planning committee 2018

Appendix K: Supervision of MSc student

Thesis title: Incidence of *Schistosoma*-host snails along River Asao, Kisumu-Homa Bay counties and the use of PCR-HRMS compared to microscopy in detection of *Schistosoma* in snails.

Appendix L: Postgraduate courses

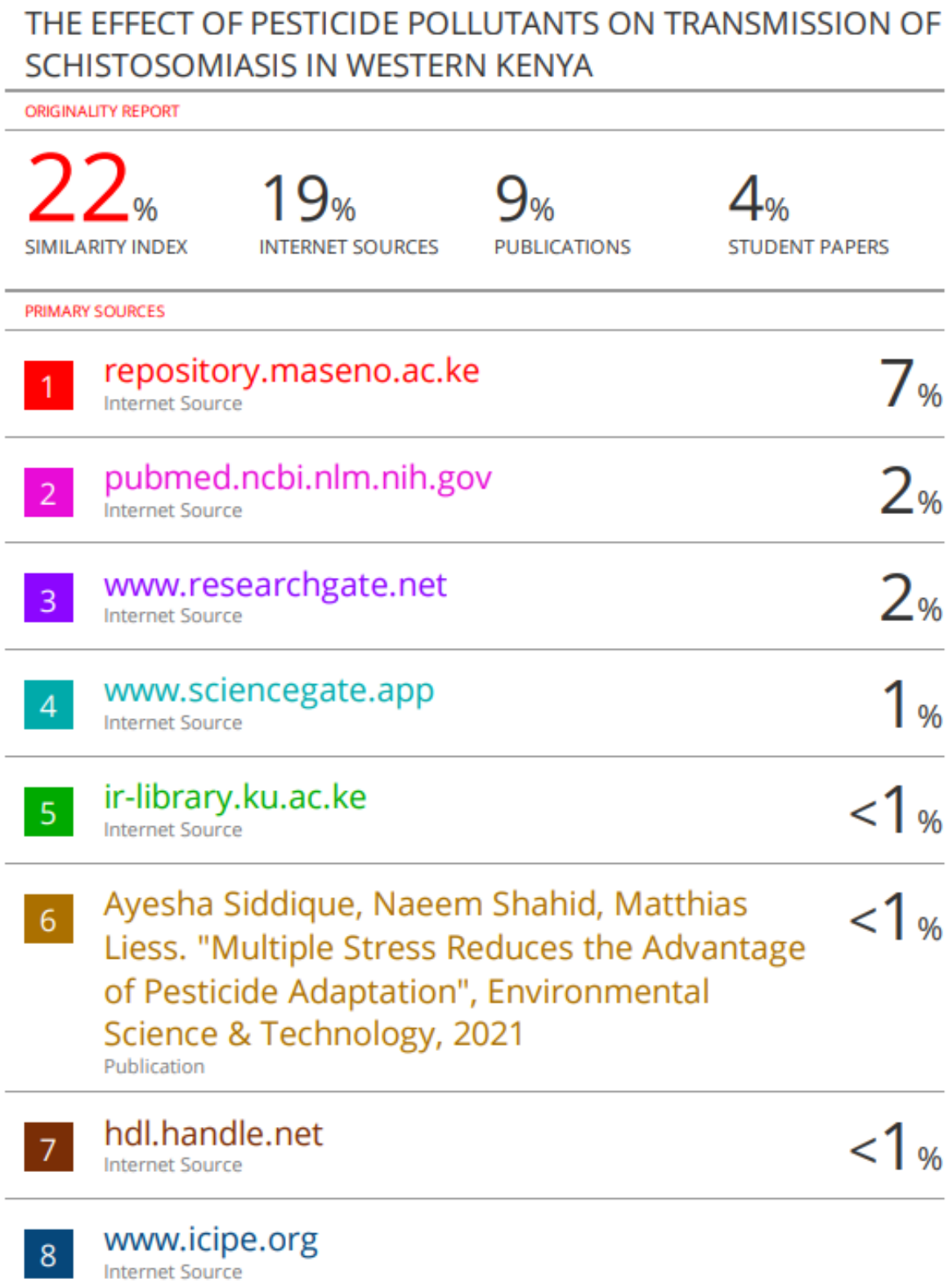
Methods of Ecotoxicology *Icipe* and Helmholtz UFZ		6th and 7th April 2022
Arthropode-borne pathogens Berlin Universität		10th March 2022
Research Methods Course NIMR – Mwanza		6th March 2020
Tropical Parasitology: Protozoans, Worms, Vectors and Human Diseases Duke University		10th February 2020
Scientific writing and publishing APHRC		8th November 2019
Genome-editing: Bio-medical applications and insect-borne disease control TreND		28th June 2019
Insect Chemical Ecology *icipe*/MaxPlanck/PennState/SLU		21st June 2019
Scientific writing and publishing *icipe*/APHRC		14th September 2018
Statistical analysis and Data analysis with R *icipe*		30th June 2017

Appendix M: Ethics certificates

CITI		2021
GHN Introduction to Clinical Research		2019

FHI 360		2019
GHN Research Ethics		2019
GHN Good Clinical Practice		2017
NIH		2017

Appendix N: Plagiarism report



THE EFFECT OF PESTICIDE POLLUTANTS ON TRANSMISSION OF SCHISTOSOMIASIS IN WESTERN KENYA

ORIGINALITY REPORT

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7	hdl.handle.net Internet Source	<1%
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