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Ultraviolet disinfection of schistosome cercariae in water

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Declaration of originality

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Copyright statement

Abstract

Schistosomiasis is a tropical disease that is contracted by skin contact with water containing cercariae, larvae of the *Schistosoma* parasite. Providing safe water for contact activities (e.g. laundry, bathing) can help reduce transmission. UV disinfection is a widely used form of water treatment and its application in low-income settings is now becoming a reality through solar-powered units. This thesis examines the effectiveness of UV LED disinfection against schistosome cercariae for use in endemic regions. A systematic review revealed that low UV fluences (5–14 mJ/cm^2 at 253.7 nm) were required to achieve a 1-$\log_{10}$ reduction in worm burden in animal hosts, but the direct effect on cercariae had not been quantified. There were insufficient published data to produce UV disinfection guidelines, therefore experiments were carried out to determine the fluence-response of *Schistosoma mansoni* cercariae at four peak wavelengths in the germicidal range (253.7 nm, 255 nm, 265 nm, and 285 nm). The morphology and motility of cercariae were studied under the microscope to determine if they were alive or dead. At the most effective wavelength (265 nm) 247 mJ/cm^2 was required to achieve a 1-$\log_{10}$ reduction in alive cercariae but this reduced to 127 mJ/cm^2 and 99 mJ/cm^2 if samples were stored for 1–3 hours post-exposure, respectively. Fluences were much higher than those required to achieve the same reduction in worm burden in previous studies and further research was needed to investigate the potential disinfection mechanisms. Using an immunological assay to detect dimer formation it was found that DNA was damaged at lower fluences (10–50 mJ/cm^2, depending on wavelength), but this damage would not be expressed until cercariae penetrate the skin and transform into schistosomula. Further research is required to confirm if cercariae are non-viable at these fluences, until then a conservative approach based on the death of cercariae is appropriate. Due to the high fluences required to kill cercariae, UV disinfection alone is unlikely to be an energy- or cost-efficient water treatment method for combatting schistosomiasis, however improvements in efficiency combined with cheaper production costs may make UV LED technology more competitive in the near future.

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List of Abbreviations

AAU Addis Ababa University, Ethiopia
ANOVA Analysis of variance
BEST Behaviour, environment, social inclusion, treatment and care
CDC Centre for Disease Control, United States
CPD Cyclobutane pyrimidine dimer
CV Coefficient of variation
DALY Disability-adjusted life year, a measure of disease burden
DNA Deoxyribonucleic acid
ELISA Enzyme-linked immunosorbent assay
FDA Fluorescein diacetate
FGS Female genital schistosomiasis
FT Food-borne trematodiasis
FWHM Full width, half maximum
GBD Global Burden of Disease Study
ICL Imperial College London, UK
LED Light-emitting diode
LP lamp Low pressure mercury arc lamp (near monochromatic)
MDA Mass drug administration
MGS Males genital schistosomaisis
MP lamp Medium pressure mercury arc lamp (polychromatic)
NHM Natural History Museum, UK
NMRI National Medical Research Institute, Tanzania
NTD Neglected tropical disease
OD Optical density
PC Preventive chemotherapy
PI Propidium iodide
RNA Ribonucleic acid
ROS Reactive oxygen species
RPL Roger Perry Laboratory
rpm Rotations per minute
SAC School-aged children
SEM Scanning electron microscopy
SODIS Solar disinfection
SPD Spectral power distribution
spp Species
STH Soil-transmitted helminthiases
UV Ultraviolet
WASH Water, sanitation, and hygiene
WHO World Health Organization
WISER Water Infrastructure for Schistosomiasis-Endemic Regions
WPE Wall plug energy

Glossary of technical terms

Dimer
A chemical compound made of two smaller and similar molecules. In the context of UV disinfection, dimer refers to the photoproducts that may be formed when nucleic acids absorb a UV photon. Dimers change the structure of nucleic acids, preventing them from being transcribed or replicated.

Fluence (mJ/cm^2)
Often used interchangeably with "UV dose", fluence is the incident radiant energy per unit area passing through an infinitely small sphere of cross-sectional area dA, from all directions. If samples are exposed to a constant fluence rate, the fluence is the product of the fluence rate and the exposure time.

Fluence rate (mW/cm^2)
The incident radiant power per unit area passing through an infinitely small sphere of cross-sectional area dA, from all directions.

Germicidal range
The range of UV light that is most effective for inactivating pathogens, 200–300 nm

Irradiance (mW/cm^2)
The incident radiant power per unit area from all upward directions on an infinitely small surface of the area dS. For a parallel and perpendicular (collimated) beam of light the irradiance is equal to the fluence rate and under the correct experimental conditions, the fluence rate applied to a water sample can be approximated using a radiometer to measure the irradiance.

1 Introduction

Schistosomiasis is a water-based neglected tropical disease (NTD) caused by parasitic worms of the genus *Schistosoma*. NTDs are a group of 20 diverse communicable diseases that primarily affect the poorest communities in tropical and sub-tropical regions, particularly where there is insufficient access to safe water, sanitation, and hygiene (WASH) and essential medicine (Hotez *et al.*, 2006). The current list of NTDs were identified by the World Health Organization (WHO) because they receive very little funding relative to the enormous health, social, and economic burdens they impose on an estimated one billion people (Molyneux, 2004). Whilst individual NTDs may not cause significant mortality relative to other diseases, collectively they are responsible for over 18 million disability adjusted life years (DALYs, GBD 2016 Collaborators, 2017), although many researchers believe the true burden is much higher (Hotez *et al.*, 2014; Herricks *et al.*, 2017; King and Galvani, 2018). Such disabling conditions, which can persist even after treatment, reduce quality of life and prevent people from accessing opportunities to succeed, such as education and work, trapping communities in a vicious cycle of poverty and disease (King and Bertino, 2008; Weiss, 2008; Conteh *et al.*, 2010; Fürst *et al.*, 2012).

Schistosomiasis affects approximately 200 million people, mainly in sub-Saharan Africa, although transmission is present in 78 countries worldwide (WHO, 2020a). Three species are responsible for the majority of infections: *Schistosoma mansoni* in Africa, the Middle East, and Latin America; *Schistosoma haematobium* in Africa and the Middle East; and *Schistosoma japonicum* in south east Asia (Colley *et al.*, 2014). Symptoms of schistosomiasis include chronic abdominal pain, anaemia, malnutrition, and impaired growth and cognitive development, particularly in children. In severe cases it can cause liver disease, kidney failure, or bladder cancer, depending on the species. Genital schistosomiasis can result in infertility and has also been linked to increase susceptibility to HIV infection, cervical cancer, and prostate cancer (Hotez *et al.*, 2019b; Kayuni *et al.*, 2019; Zirimenya *et al.*, 2020). Infection occurs through skin contact with water (e.g. through bathing or doing laundry) containing the parasite larvae, known as cercariae, which are produced by asexual reproduction within intermediate freshwater snail hosts. Schistosome cercariae penetrate human skin and, once inside the body, develop into worms that live in the blood vessels of the urogenital or intestinal systems, depending on the species. Adult worm pairs produce eggs that are released in the urine or faeces. Where there is no access to sanitation or inadequate containment of human waste eggs may be released into water bodies, enabling infection of freshwater snails and continuation of the life cycle.

The deworming medication praziquantel is an affordable and effective treatment and preventive chemotherapy is central to schistosomiasis control programmes (WHO, 2002). This has been successful in reducing the global health burden of schistosomiasis (Fenwick *et al.*, 2009; Rollinson *et al.*, 2013) but treatment cannot prevent reinfection if contact with cercariae infested water continues. As a result, prevalence remains high in communities that are reliant on surface water for daily domestic, recreational, or occupational activities. Other complementary control strategies include snail control, WASH provision, and behaviour change. Snail control can interrupt schistosomiasis transmission by reducing the number of intermediate snail hosts, but chemical mollusciciding is expensive and can be

toxic to fish (King and Bertsch, 2015; Huang *et al.*, 2013), so it is best used in a targeted manner (Coelho and Caldeira, 2016). Improving access to sanitation can reduce the number of schistosome eggs reaching snail habitats, however as only a small number of eggs are required to maintain the life cycle, there is unlikely to be an effect on schistosomiasis transmission unless there is universal uptake in the community (Secor, 2014). Similarly, providing access to safe water sources could prevent exposure to schistosome cercariae, but will only be fully effective if contact with surface water also stops (Kholy *et al.*, 1989), requiring health education and behaviour change campaigns.

Schistosomiasis is targeted for global elimination as a public health problem by 2030, defined as less than 1% heavy infections (≥ 400 eggs per gram of faeces or ≥ 50 eggs per 10 mL of urine) in the school-aged population (WHO, 2002; WHO, 2020b). However, due to the complexity of the disease and rate of infection, current control strategies that focus on preventive chemotherapy can only have a temporary effect on transmission (Guidi *et al.*, 2010; Rollinson *et al.*, 2013). A multisectoral approach making use of complementary interventions that are tailored to suit each community's requirements may be more sustainable and in recent years there have been growing calls for improving collaboration between WASH and NTD programmes (in particular Lancet Editorial Board (2012), Freeman *et al.* (2013), Campbell *et al.* (2014), Nakagawa *et al.* (2015), Boisson *et al.* (2016), and Boisson *et al.* (2021)). In 2015 the WHO published its global strategy for WASH and NTDs, highlighting that WASH interventions have broad public health benefits and the potential to accelerate many NTD control initiatives at once and yet there is rarely collaboration between these programmes (WHO, 2015; Boisson *et al.*, 2016). To help combat this, the WHO published "WASH and Health working together", a toolkit for integrating WASH and NTD programmes based on the BEST (behaviour, environment, social inclusion, treatment and care) framework that was developed by NTD NGO Network (WHO, 2018b). As a result of these publications and increasing evidence of the impact of WASH on NTDs (Stocks *et al.*, 2014; Strunz *et al.*, 2014; Grimes *et al.*, 2016; Emerson *et al.*, 2020) the momentum behind WASH and NTD collaboration has continued to grow. The latest NTD roadmap places a stronger emphasis on integrated and multisectoral control strategies than ever before with cross-cutting targets that are aligned with the Sustainable Development Goals (SDG), including universal WASH access (WHO, 2020b).

Access to sanitation and clean water, and promotion of safe water practices are all key interventions under the behaviour and environmental components of the BEST framework for schistosomiasis control (WHO, 2018b). However studies have shown that water interventions may have the strongest impact on schistosomiasis transmission as they reduce contact with cercariae and therefore directly reduce risk of infection (Jordan *et al.*, 1975; Grimes *et al.*, 2016; Gichuki *et al.*, 2019). Whilst standpipes and boreholes are often installed in WASH programmes to provide drinking water, they may not meet the needs of the community for other activities such as laundry and bathing, and contact with contaminated surface water sources may continue (Kholy *et al.*, 1989). In these instances water that has been collected from contaminated water bodies should be treated before use (e.g. by filtration, chlorination, solar or ultraviolet (UV) disinfection) to remove or inactivate cercariae.

Solar disinfection (SODIS) is widely used to treat drinking water in low-income regions (McGuigan *et al.*, 2011; Preez *et al.*, 2011; McGuigan *et al.*, 2012) and UV disinfection with mercury arc lamps is now also becoming a reality through solar-powered units (Brownell *et al.*, 2007; Opryszko *et al.*, 2013; Hunter *et al.*, 2013). These solutions are chemical-free and have no risk of over-dosing or producing regulated

by-products (such as trihalomethanes or haloacetic acids produced by chlorination (Reckhow and Singer, 1990)). The emerging technology of UV light-emitting diodes (UV LEDs), which are mercury-free, durable, and very well suited to point-of-use solutions, have the potential to further revolutionise water treatment in low-income regions. There are a number of ways SODIS or UV disinfection could be harnessed for schistosomiasis control programmes, providing safe water for contact activities at an individual, household, or community scale (Figure 1.1). However, there are currently no formal guidelines for treating water against schistosome cercariae, making it difficult to implement treatment systems and WASH programmes that directly target schistosomiasis transmission (Campbell *et al.*, 2018).
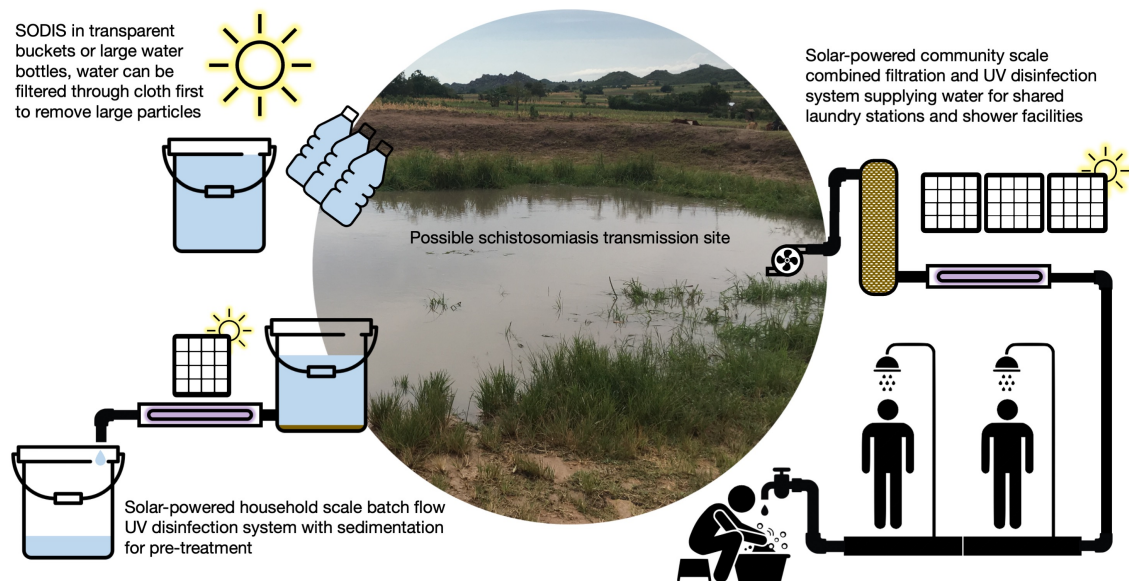
Figure 1.1 Possible strategies for implementing solar or ultraviolet disinfection to provide safe water for contact activities in schistosomiasis endemic regions. Water could be collected by hand then treated by small household systems, or it could be pumped from local rivers or streams to supply community facilities such as laundry stations and showers. Depending on the level of treatment the water could also be used for drinking. Composite image produced in Microsoft® PowerPoint for Mac version 16.56.

1.1 The WISER project

The "Water Infrastructure for Schistosomiasis-Endemic Regions" (WISER) research project was set up to address the knowledge gaps regarding water treatment processes against schistosome cercariae. Funded by the Engineering and Physical Sciences Research Council through the Global Challenges Research Fund (grant number EP/P028519/1) with a budget of 1.5 million GBP, the project ran from May 2017 to April 2021. It was a collaboration between engineers, synthetic biologists, parasitologists, and social scientists at four research institutions: Imperial College London (ICL, UK), the Natural History Museum (NHM, UK), Addis Ababa University (AAU, Ethiopia), and the National Institute for Medical Research (NIMR, Tanzania). The project had three research objectives: 1) determine the effectiveness of water treatment processes against schistosome cercariae; 2) develop a cheap and rapid biosensor to detect the presence of cercariae in water; and 3) evaluate the effectiveness of education programmes for promoting safe water behaviour and understand non-technical barriers to implementing water infrastructure. This thesis addresses part of objective 1.

1.2 Aim and objectives

The overall aim of this thesis was to determine the effectiveness of UV disinfection against schistosome cercariae in water and examine the photobiological mechanisms involved. This was achieved through a systematic review and a series of UV disinfection experiments that determined the fluence- (or UV dose, refer to the glossary and Section 2.4.6 for definitions) response of *S. mansoni* cercariae and investigated possible UV damage mechanisms. These three objectives and their respective research questions can be summarised as follows:

1. Systematic review

   - Review existing literature on UV sensitivity of WASH-related helminths, including schistosome cercariae. What helminths have been studied? What UV wavelengths have been tested? What methods were used to establish viability and are they comparable?
   - Determine which helminths may be more susceptible to this form of water treatment, based on available studies. Do the data suggest schistosome cercariae are susceptible to UV disinfection? Are the data reliable?
   - Identify gaps in research which will inform future studies regarding the proper use of UV and SODIS for minimising the spread of these diseases via water in low-income regions.

2. UV fluence-response

   - Examine the direct effect of UV disinfection on *S. mansoni* cercariae in water, using established protocols for fluence determination.
   - Determine the fluence-response at each wavelength. How does this compare to the literature?
   - Which wavelength is most effective? What does this suggest about the inactivation mechanism?

3. UV damage mechanism

   - Investigate possible UV damage mechanisms using an immunological assay to assess UV-induced DNA damage in *S. mansoni* cercariae.
   - Can UV-induced DNA damage be measured? Is it possible to determine a fluence-response?
   - Does the fluence-response vary with wavelength? What does this demonstrate about the UV damage mechanisms in schistosome cercariae?

1.3 Publications and presentations

Much of the work included in this thesis has been published in two peer-reviewed publications:

- Hazell, L., Braun, L., Templeton, M. R. (2019) Ultraviolet sensitivity of WASH (water, sanitation, and hygiene) related helminths: A systematic review. *PLOS Neglected Tropical Diseases* 13(9) DOI:10.1371/journal.pntd.0007777
- Hazell, L., Allan, F., Emery, A. M., Templeton, M. R. (2021) Ultraviolet disinfection of *Schistosoma mansoni* cercariae in water. *PLOS Neglected Tropical Diseases* 15(7) DOI:10.1371/journal.pntd.0009572

Throughout the course of my doctorate I also contributed to the following publications:

- Braun, L., Hazell, L., Webb, A. J., Allan, F., Emery, A. M., Templeton, M. R. (2020) Determining the viability of *Schistosoma mansoni* cercariae using fluorescence assays: An application for water treatment. *PLOS Neglected Tropical Diseases* 14(3) DOI:10.1371/journal.pntd.0008176

- Blyth, J., Templeton, M. R., Court, S. J., Luce, C., Cairns, W. and Hazell, L. (2021) Assessment of indigenous surrogate microorganisms for UV disinfection dose verification. *Water and Environment Journal* DOI:10.1111/wej.12722

- Blyth, J., Hazell, L., Templeton, M. R. (2021) Immunological detection of thymine dimers in indigenous genomic DNA from pre-disinfection drinking water as an ultraviolet disinfection dosimeter. *Environmental Science: Water Research & Technology* 7(11) DOI:10.1039/D0EW00939C

Some of this research was presented at international conferences:

- 2019 Annual Meeting, ASTMH, Maryland, USA. Ultraviolet disinfection of schistosome cercariae in water using UV-C LEDs.

- 2021 Schistosomiasis Research Innovation Meeting, GSA, Online. Effectiveness of ultraviolet disinfection against schistosome cercariae in water.

1.4   Thesis structure

Chapter 2 presents a background to schistosomiasis and UV disinfection. The UV sensitivity of WASH-related helminths is systematically reviewed in Chapter 3. Chapter 4 presents the UV disinfection experiments that enabled the determination of the fluence-response of *S. mansoni* cercariae at four wavelengths and in Chapter 5, the UV disinfection mechanism is investigated in more detail through the use of immunological assays to detect DNA damage. The discussion is presented in Chapter 6 and final conclusions are drawn in Chapter 7. References and appendices can be found at the end of the thesis.

2 Background and literature review

2.1 Human schistosomes

Schistosoma is a genus of trematodes, a class of helminths (parasitic worms) that are defined by a complex life cycle involving at least two hosts, a definitive vertebrate host in which they reproduce sexually and an intermediate host (usually molluscan) where asexual reproduction occurs. The life cycles of the three major human schistosomes, *S. haematobium, S. mansoni* and *S. japonicum*, are similar (Figure 2.1), with the main differences being the intermediate host species, distribution within the definitive human host, and geographic location. Free-swimming schistosome cercariae infect humans by penetrating the skin, once inside they develop into adult worms (schistosomes) which mate and produce eggs. Eggs are released from the body in faeces and urine, they hatch in freshwater releasing miracidia which seek out and infect species specific aquatic snails. Inside the intermediate snail host the parasite reproduces asexually to form cercariae which are shed back into the water, completing the life cycle.
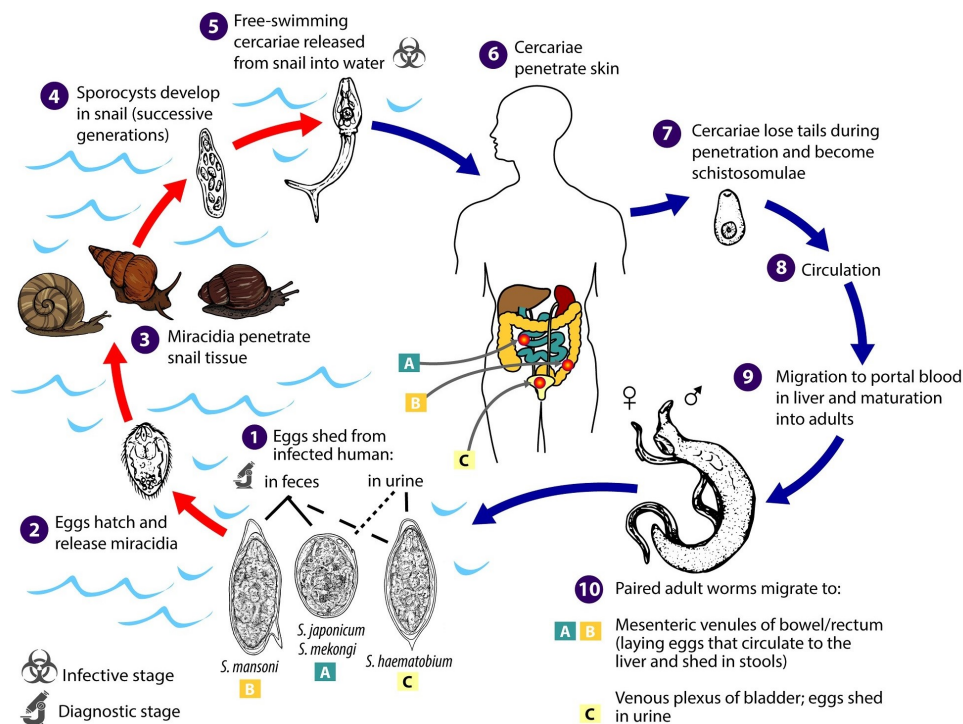
Figure 2.1 The life cycle of the three main human schistosomes. Adapted from CDC (2012).

2.1.1 Schistosomes

Unusually for helminths, schistosomes are dioecious, meaning they have two distinct sexes. The worms mature into adults in the hepatic portal system, where mate finding also occurs (Popiel, 1986). Mate pairing is required for the worms to complete maturation, and for migration from the hepatic portal system to the venous plexus of the bladder (*S. haematobium*) or mesenteric venules of the bowels (all other species) where the worms live *in copula* (Armstrong, 1965; Erasmus, 1973; Popiel, 1986). The thinner female (10–20 mm long, depending on species) is held with in the gynaecophoric canal of the
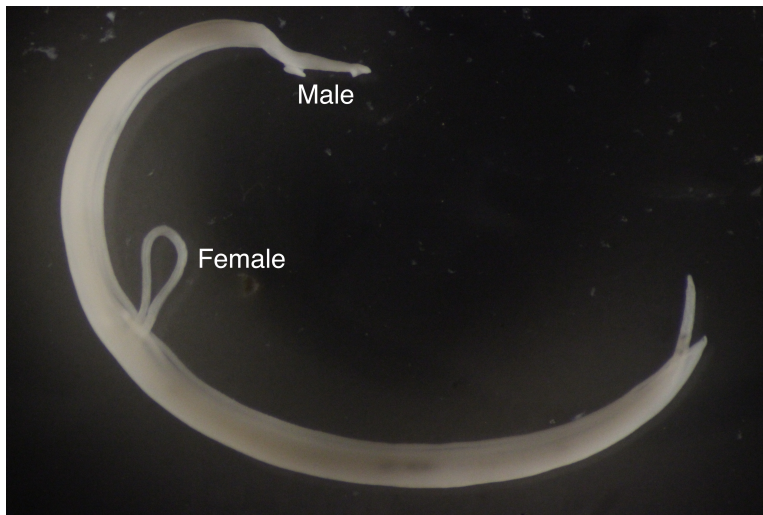
(a)

(b)

Figure 2.2 (a) A pair of adult *Schistosoma bovis* worms which are larger but physiologically similar to the human schistosomes. *Schistosoma* means "split body" in Latin, a reference to the gynaecophric canal in which the male holds the the thinner female. Image taken by Dr Aidan Emery © The Natural History Museum, reproduced under license CC BY 4.0. **(b)** Over 1500 *Schistosoma mansoni* worms removed from an 18 year old patient in 1970 by live surgical perfusion. From Colley and Secor (2007).

male (6–13 mm long, Lewis, 1998); here the female produces eggs for the male to fertilise (Figure 2.2). A physical association between male and female is required for egg production to occur, and studies have shown that egg-laying females separated from their male partners will stop producing eggs and regress into an immature state. This process is reversible if females are able to pair again with males, and egg laying can resume even months after regression (Erasmus, 1973; Popiel *et al.*, 1984). Mate pairing is generally considered to be monogamous, although schistosomes have been shown to change partners (Tchuem Tchuenté *et al.*, 1996; Beltran and Boissier, 2008; Steinauer, 2009). Adult pairs are estimated to live for 3–10 years on average (Fulford *et al.*, 1995; Gryseels *et al.*, 2006), but they can survive for decades (Hall and Kehoe, 1970; Harris *et al.*, 1984). Schistosomes live in the blood vessels and feed by ingesting blood and by absorbing nutrients directly through the body surface; active females can consume up to four times their body volume in blood each day (Skelly *et al.*, 2014).

2.1.2 Egg production

The prepatent period in humans (the time between skin penetration and egg production) is 4–5 weeks for *S. haematobium* and *S. mansoni* and 6–7 weeks for *S. japonicum* (Sturrock, 2001b). The size and shape of eggs also varies between species (Figure 2.3), *S. haematobium* and *S. mansoni* eggs are approximately 150 μm long, with a terminal and lateral spine, respectively. *S. japonicum* eggs are smaller, approximately 90 μm long and more rounded than other species, with a smaller, less conspicuous, lateral spine (Manson-Bahr and Fairley, 1920; MCD International, 2010). The function of the spine remains unclear, although it has been suggested that it aids attachment to the lining of the capillary walls, where the eggs are deposited by female worms (Schwartz and Fallon, 2018). From here eggs are either swept away by the bloodstream into various tissues and organs, or they pass through the capillary walls into the epithilium before being released into the bladder or intestines and excreted in urine (*S. haematobium*) or faeces (all other species).
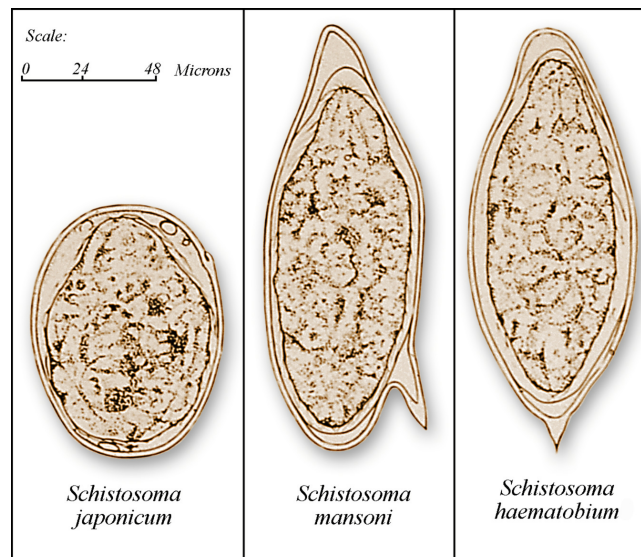
Figure 2.3 Eggs of the three main human schistosomes. Adapted from Melvin (1973).

Eggs that become trapped in the body in organs such as the liver and spleen induce an immune response around the eggs (McManus *et al.*, 2018). This inflammation and subsequent fibrosis causes much of the morbidity associated with schistosomiasis (Section 2.2.3), but granuloma formation around eggs that remain attached to the capillary walls is also the main mechanism that facilitates passage of *S. mansoni* eggs from the blood vessels into the intestines (Hams *et al.*, 2013; Schwartz and Fallon, 2018). This relationship between egg and host, and manipulation of the immune system, demonstrates how perfectly-adapted schistosomes are to infect humans (Hams *et al.*, 2013).

Depending on the species, schistosome pairs can produce hundreds (*S. haematobium* and *S. mansoni*) or thousands (*S. japonicum*) of eggs per day (Table 2.1) but fecundity is density-dependent, with fewer eggs being produced per worm pair in high-intensity infections (Medley and Anderson, 1985; Neves *et al.*, 2021). Detection of viable eggs in stool or urine samples is the diagnostic standard for schistosomiasis, although a negative result cannot rule out infection, as low egg counts may be missed (Wilson *et al.*, 2006). A high-intensity infection is defined as ≥ 400 eggs per gram of faeces for *S. mansoni* whilst ≥ 50 eggs/10 mL of urine is deemed a high-intensity infection of *S. haematobium* (WHO, 2002).

After being released from the female, eggs can survive for 1–2 weeks whether inside the body or excreted (Colley *et al.*, 2014), although in dry environments desiccated eggs may die within a few days (Chandiwana, 1986). The most likely route of *S. haematobium* eggs into water bodies is through direct urination, mainly by children (Rollinson, 2009). Whilst this is common behaviour in all children, it has been suggested that the pathology of urinary schistosomiasis may enhance the opportunity for eggs to be released into water, as it reduces bladder elasticity (and therefore capacity) (Sayegh and Dimmette, 1956) and causes pain during urination which children may seek to alleviate by urinating in cool water (Rudge *et al.*, 2008). Direct defecation into water bodies may be considered taboo in some cultures (Sow *et al.*, 2008) however it can be common in fishing communities, as fishers spend long hours on boats with no access to sanitation facilities (Samikwa *et al.*, 2019), and some believe open defecation into the lake water is beneficial for fish growth (Berling *et al.*, 2013). Open defecation sites may be located near water bodies as they provide the opportunity for washing after defecation and vegetation for privacy (Cheesmond and Fenwick, 1981; Sow *et al.*, 2004; Grimes *et al.*, 2015).

Such sites enable faeces to be washed into the water during rain or flooding, or it may be trodden into the water by people and animals (Chandiwana, 1986). Another important transmission route is eggs washing off of soiled clothing when laundering or washing off from the body during hand washing, bathing, or swimming (Rollinson *et al.*, 2013; Grimes *et al.*, 2015). In particular, anal-cleansing after defecation is likely to be a major contributor to *S. mansoni* transmission (Sow *et al.*, 2008). Eggs may also be deposited into water bodies by reservoir hosts, although this is only thought to be a significant transmission route for *S. japonicum* which is a zoonotic species, known to infect at least 46 species of mammals (He *et al.*, 2001; Rollinson *et al.*, 2013; Grimes *et al.*, 2015).

2.1.3 Miracidia and the intermediate host

Eggs hatch within minutes of contact with warm freshwater (Samuelson *et al.*, 1984), each one releasing a single miracidium. *S. mansoni* and *S. haematobium* miracidia are 130–170 µm long, whilst *S. japonicum* are much smaller at approximately 80 µm (Sturrock, 2001b). They are covered in ciliated epidermal cells that are used for swimming, enabling them to move quickly at 2–3 mm/sec in freshwater (Plorin and Gilbertson, 1981). Miracidia are non-feeders and they must find a suitable snail host in order to survive. Prah and James (1977) found *S. mansoni* and *S. haematobium* miracidia survived for 9–15 hours in freshwater at 18–22 °C, whilst Anderson *et al.* (1982) suggest that the maximum life expectancy of *S. mansoni* miracidia is 16 hours at 25 °C.

Figure 2.4 A *Schistosoma mansoni* miracidium, approximately 150 µm in length. Image taken by Dr Aidan Emery © The Natural History Museum, reproduced under license CC BY 4.0.

Each schistosome species has specific intermediate snail host species: *Bulinus* spp. for *S. haematobium*, *Biomphalaria* spp. for *S. mansoni*, and *Oncomelania* spp. for *S. japonicum* (Figure 2.5). Miricidia respond to environmental cues such as light and temperature to locate snail habitats and are attracted to sail hosts by chemical stimuli that are released in the snails' mucous secretions (MacInnis, 1965; Haas *et al.*, 1995; Jamieson *et al.*, 2017). Secretions differ between snail species, enabling miracidia to locate their specific snail hosts and they have also been shown to prefer uninfected snails over patent ones (Kalbe *et al.*, 1996; Allan *et al.*, 2009).

Miracidia infect snails by penetrating their flesh. Infectivity is dependent on the age of miracidia and

Figure 2.5 The intermediate snail hosts of the main human schistosomes, from left to right: *Bulinus* spp. *Biomphalaria* spp., and *Oncomelania* spp. Shell diameter of *Bulinus* spp. 5 mm. From Lewis *et al.* (2008), reproduced under license CC BY 4.0.

the water temperature, with the rate of infection of *S. mansoni* peaking at 25 °C (Anderson *et al.*, 1982). Once inside the snail, miracidia transform into mother sporocysts which undergo asexual reproduction to produce up to 600 secondary sporocysts from one miracidium (Jamieson *et al.*, 2017). Secondary sporocysts migrate to the digestive glands of the snail and complete their transformation into cercariae. Cercarial formation in secondary sporocysts is also asexual, therefore one miracidium can produce thousands of cercariae over the lifetime of an infected snail (Gryseels *et al.*, 2006). The prepatent period in snails (the time between miracidium infection and release of cercariae) is temperature dependent and varies between species. *S. mansoni* and *S. haematobium* have similar prepatent periods at approximately 18–30 days at 25–33 °C (Stirewalt, 1954; Kalinda *et al.*, 2017). For *S. japonicum* the prepatent period can be up to 90 days at 24–28 °C (Moloney *et al.*, 1987). Schistosome infection has been shown to reduce growth and fecundity in host snails and increase mortality, with snail lifespans decreasing with increasing intensity of miracidia infection (Etges and Gresso, 1965; Fryer *et al.*, 1990; Woolhouse, 2016).

2.1.4 Cercariae

Emergence of cercariae from snail hosts is diurnal and stimulated by exposure to light (Figure 2.6). Depending on the species and intensity of infection, snails can shed tens or hundreds of cercariae each day (Table 2.1). Cercariometry studies that use filtration to determine the temporal and spacial distribution of schistosome cercariae in natural water bodies have reported ranges from 0.1 to over 100 cercariae/litre, depending on the location (Rowan, 1958; Kloos *et al.*, 1982; Prentice and Ouma, 1984; Ouma *et al.*, 1989; Muhoho *et al.*, 1997; Aoki *et al.*, 2003). Concentrations also vary with the season and the time of day. Seasonal variation is proportional to the number of infected snails, which increase during rainy seasons (Muhoho *et al.*, 1997), whilst diurnal variation is dependent on a number of interactive conditions such as the cercarial shedding pattern, water flow, water depth, and proximity to snail habitat (see Section 2.2.2). As this thesis investigates the effect of UV disinfection on the human infectious stage of the life cycle, the following sections explore the anatomy and behaviour of schistosome cercariae in more detail.
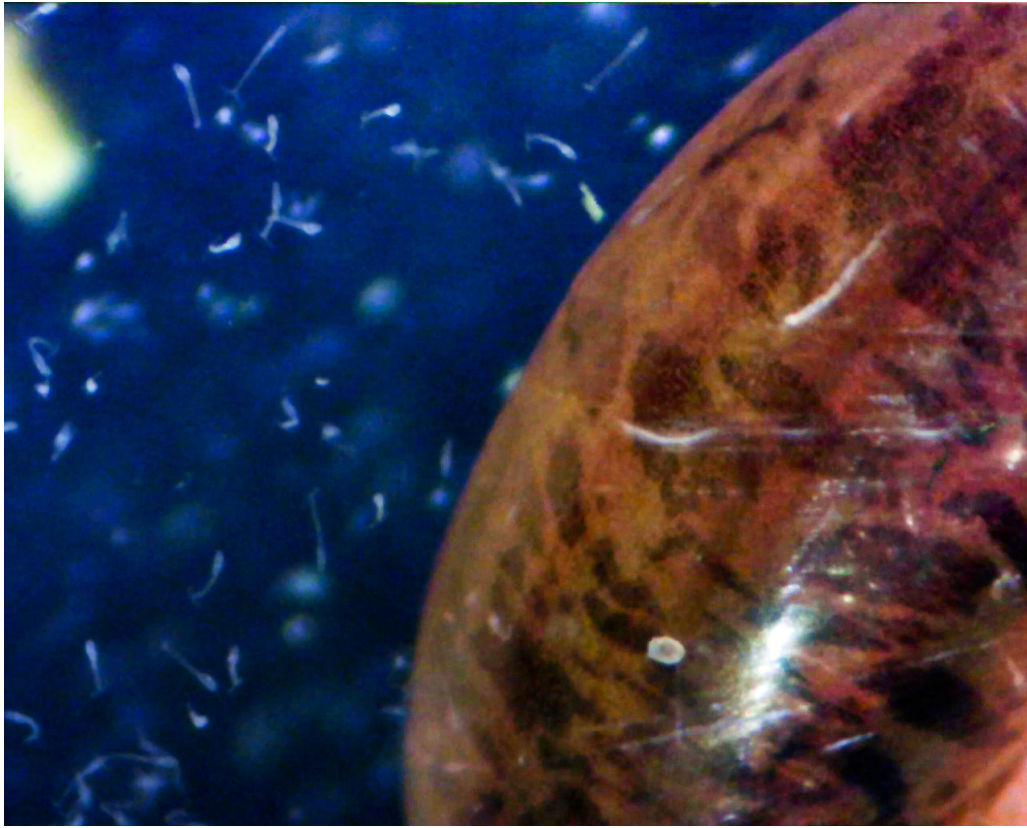
Figure 2.6 *Schistosoma mansoni* cercariae emerging from patent *Biomphalaria glabrata* snail.

Table 2.1 Characteristics of the three main human schistosome species. Some vary with environmental conditions and intensity of infection (l×d = length × diameter.

	S. mansoni	*S. haematobium*	*S. japonicum*	References
Disease	Intestinal	Urogenital	Intestinal	
Snail host	*Biomphalaria* spp.	*Bulinus* spp.	*Oncomelania* spp.	
Prepatent period in humans (weeks)	4–5	4–5	6–7	Sturrock, 2001b
Prepatent period in snails (days)	18–30	18–30	90	Stirewalt, 1954; Moloney *et al*, 1987; Kalinda *et al*, 2017
Egg size & shape	150 µm lateral spine	150 µm terminal spine	90 µm lateral spine	Manson-Bah and Fairley, 1920
Eggs per day	250–550	500–1000	3500	Moore and Sandground, 1956; Ayad, 1974
Miracidia size (l)	130–170 µm	130–170 µm	80 µm	Sturrock, 2001b
Cercariae size (l×d)	374×60 µm	398×64 µm	250×40 µm	Manson-Bah and Fairley, 1920
Cercariae per day	250–600	200	15–160	Ayad, 1974; Jordan and Webbe, 1982

2.1.4.1 Anatomy

Schistosome cercariae are free-swimming larvae approximately 250–400 μm long and 40–60 μm wide, depending on the species (Table 2.1). They have two distinct parts, the head (also referred to as the body) and a bifurcated tail. They are complex microorganisms, formed of an estimated 1000 cells, the bulk of which are muscle cells, that are used to contract the head and tail during swimming and skin penetration (Dorsey *et al.*, 2002). The main features of the cercarial head are the the oral sucker, acetabulum (ventral sucker), and the excretory glands (Figure 2.7a). The head also contains all of the organs found in the adult worm. Some, such as the oesophagus, are well developed even though they do not appear functional until transformation into schistosomula. Others are only partially developed or present as germ cells, such as with the reproductive organs (Dorsey *et al.*, 2002; Stirewalt, 1974).

The oral sucker and acetabulum are used during skin exploration and penetration. They attach alternately to the skin in adhesive secretions, enabling the cercariae to crawl across the surface and search for an entry point (Haas and Haeberlein, 2009). Once located, the acetabulum acts as an anchor, whilst the oral sucker burrows into the skin (Dorsey *et al.*, 2002; Stirewalt and Kruidenier, 1961). A large volume of the head is taken up by the pre- and post-acetabular glands, of which there are two and three pairs, respectively (Figure 2.7b). The glands are named by their location on the head relative to the acetabulum. Stimulation by skin chemicals causes the glands to release their secretions through ducts which terminate in apertures located on the rim of the oral sucker (Shiff *et al.*, 1972). These glands are emptied during skin exploration and penetration, and are not present in adult schistosomes (Stirewalt and Fregeau, 1966).

The main function of post-acetabular gland secretions is thought to be adhesion. Both suckers attach to the skin in the adhesive mucus as cercariae crawl over the skin, and swimming cercariae have been observed depositing mucus from the oral sucker onto the acetabulum to ensure adhesion (Stirewalt, 1965). It has also be suggested that the post-acetabular secretions aid penetration and transformation into schistosomula, by directing enzymes, protecting the surface of the cercariae, and assisting tail detachment (Stirewalt and Kruidenier, 1961; Stirewalt and Walters, 1973; Howells *et al.*, 1974). Pre-acetabular secretions contain enzymes which aid skin penetration by degrading elements of the epidermis, providing a pathway for the cercariae (Campbell *et al.*, 1976; Stirewalt and Kruidenier, 1961). They also contain calcium, the function of which is not clear but it is thought to either aid penetration or adhesion, protect the cercarial surface in the presence of enzymes, or it may be involved in the chemical changes to the cercarial surface during transformation into schistosomula (Stirewalt, 1974). The head and tail are joined at a ball-and-socket-like junction, surrounded by a collar. The tail is often considered a temporary organ that serves to propel the cercariae through the water, and is detached during skin penetration (Dorsey *et al.*, 2002). It is very muscular with an excretory duct running along its length, emptying at two excretory pores in each of the tail forks. The entire organism is covered with a 1–2 μm thick fibrillar mesh known as a glycocalyx, which is formed late in the development of cercariae (Caulfield *et al.*, 1988). The glycocalyx is shed during transformation to schistosomula, at which point the organism is no longer water tolerant and it has therefore been suggested that this carbohydrate-rich coating protects against osmotic shock, enabling cercariae to survive in fresh-water (Morris, 1971; Stirewalt, 1974; Howells *et al.*, 1975). Beneath the glycocalyx is the tegument (0.1–0.5 μm thick), a cytoplasmic structure that is bound by a trilaminate surface membrane (0.7–10 nm) that is contiguous with the subtegumental cells (Morris, 1971). Thin, pointed spines, approximately

(a)

Oral Sucker

Mouth

Gland ducts (dark blue)

Pre-acetabular glands (light blue)

Acetabulum (pink)

Post-acetabular glands (light blue)

Glycocalyx
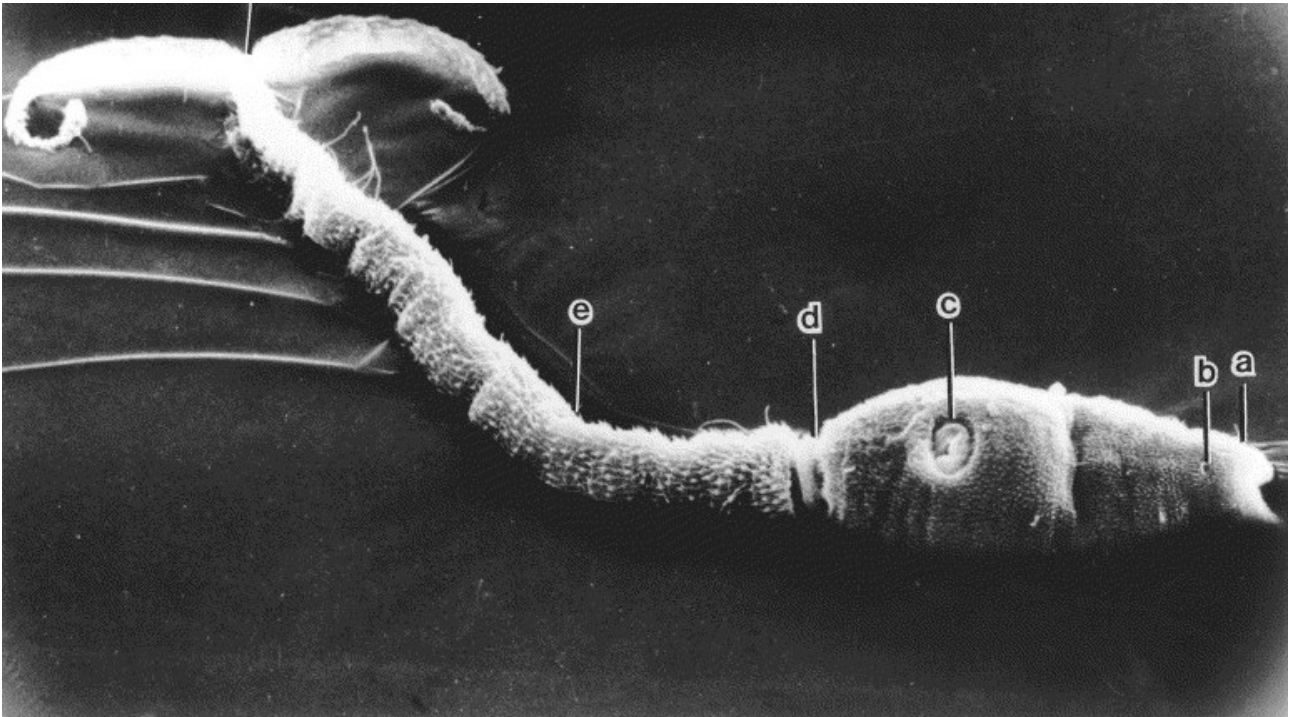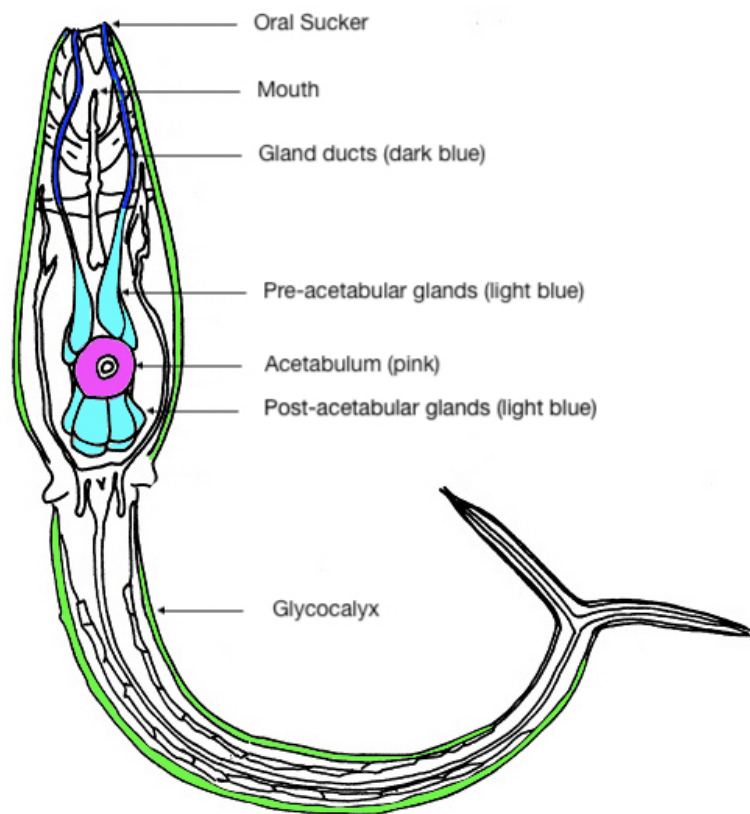
(b)

Figure 2.7 (a) A scanning electron micrograph of a *Schistosoma mansoni* cercaria with visible spines covering the head and tail, approximate length 500 μm. Main features are a) oral sucker, b) mouth, c) acetabulum, d) head-tail junction, e) bifurcated tail. **(b)** An anatomical diagram of a *Schistosoma mansoni* cercaria. Both adapted from Dorsey *et al.* (2002) with permission.

1 µm long cover the surface of the cercariae, pointing towards the tail (Dorsey *et al.*, 2002). They are more concentrated on the acetabulum than the rest of the surface and are thought to aid skin penetration by ensuring forward movement. Over 70 sensory papillae, cover the head of the cercariae, projecting from the body or in the form of cilliated pits in the tegument (Short and Cartrett, 1973). They are part of the cercarial nervous systems and are thought to act as receptors for mechanical, chemical, and photo-stimulants, aiding host finding (Short and Gagné, 1975). The tegument is also present on the schistosomula and adult schistosomes, although some structural changes occur as the parasite adapts to living inside the host tissue (Morris, 1971; Mohammed, 1999).

2.1.4.2 Life span

Like miracidia, cercariae are non-feeders. They are shed from the intermediate snail host with a finite glycogen store which is used during host finding and skin penetration (Lawson and Wilson, 1980). Life span decreases with increasing water temperature; at 15–35 °C cercariae can survive for approximately 10–40 hours, but this decreases to less than 6 hours at 40–45 °C and in temperatures above 50 °C death occurs almost instantly (Lawson and Wilson, 1980; Braun *et al.*, 2018). Between 15 and 35 °C death is thought to be as a result of ageing caused by glycogen depletion, but at higher temperatures death is believed to be caused by thermal intolerance (Lawson and Wilson, 1980). Low water temperatures have been shown to extend the lifespan of cercariae to up to 100 hours at 5–6 °C but this also has a negative impact on infectivity and the viability of schistosomula (Krakower, 1940).

Glycogen levels in cercariae decrease exponentially post emergence, but initial levels can vary significantly (Bruce *et al.*, 1969; Lawson and Wilson, 1980). In one study the mean glycogen levels varied from 4.78 ng to 14.15 ng in cercariae shed from different batches of snails (Lawson and Wilson, 1980). This variation was thought to be as a result of the nutritional condition and level of infection in the snail. Total glycogen levels do not directly correlate with infectivity and cercariae can remain highly infective for up to 9 hours post emergence, despite the exponential decrease in glycogen, and even older non-swimming cercariae are able to penetrate the skin (Lawson and Wilson, 1980; Whitfield *et al.*, 2003a). This could be because glycogen stored in the body is used at a slower rate than glycogen stored in the tail, so whilst cercariae may not be capable of spontaneous swimming they may still have sufficient energy to penetrate the skin, if they are able to find a human host (Lawson and Wilson, 1980).

External factors such as light exposure and pH (5.5–8.4) do not appear to affect the lifespan of cercariae (Krakower, 1940; Jones and Brady, 1947a), however water quality has been found to have an impact on their survival. Cercariae are killed within four hours in deionized or distilled water whereas adding optimal levels of sodium chloride and glucose to water increases the longevity of cercariae (Frick and Hillyer, 1966; Bruce *et al.*, 1969; Asch, 1975).

2.1.4.3 Host finding

Cercariae swim tail first, rapidly thrusting their forked tail side-to-side giving the appearance of spinning. Each species has a slightly different swimming pattern, *S. japonicum* cercariae use the acetabulum to cling to the water surface without swimming for minutes at a time, whilst *S. mansoni* and *S. haematobium* tend to swim in constant bursts of movement, relaxing and sinking for only a few

seconds before swimming again (He *et al.*, 2002; LoVerde, 2019).

Cercariae are known to be phototactic and are attracted to the surface of shallow water, where they are more likely to find a host to infect (Short and Gagné, 1975; McKerrow and Salter, 2002). When kept in the dark *S. mansoni* cercariae will become immobile and sink to the bottom of a container, before quickly resuming swimming when re-exposed to light (Hazell *et al.*, 2021). They are also sensitive to a number of mechanical and chemical stimuli, such as water turbulence, temperature gradients, and fatty acids present on human skin (Haas, 1994; Haas, 2003; He *et al.*, 2005). However whether cercariae are able to host-orientate and actively swim towards such stimuli, or if they simply modify their behaviour (e.g. swim for longer between rest periods, change direction more frequently) to increase the chance of finding a host is still being debated (Cohen *et al.*, 1980; McKerrow and Salter, 2002; Curwen and Wilson, 2003; Haeberlein and Haas, 2008; Brachs and Haas, 2008). In any case, *S. mansoni* cercariae appear to be more responsive to chemical stimuli, whilst *S. haematobium* are more responsive to temperature changes, and can respond to thermal gradients as low as 0.03 °C/mm (Haas *et al.*, 1994). *S. japonicum* have a more passive, energy saving, host finding method. By clinging to the surface they are transferred to the host when it withdraws from the water (Haas *et al.*, 1987; He *et al.*, 2005). This may reflect the fact that *S. japonicum* can infect a broad range of mammals, and therefore do not need host-specific stimuli (Haas *et al.*, 1987).

2.1.4.4 Skin penetration

Once a host has been located, cercariae switch from tail-first to head-first movement. Thermal gradients, fatty acids, and other compounds that are present on human skin induce the cercariae to attach to the surface and release their gland contents (Shiff *et al.*, 1972; Haas, 1976; Haas *et al.*, 1987; Haas *et al.*, 1997). Cercariae explore the skin for an entry site such as a wrinkle or follicle, although they are also able to penetrate smooth skin. Aided by cercarial proteases, they enter the epidermis within minutes (Haas and Haeberlein, 2009).

Ghandour (1976) found the optimum temperature of cercarial penetration to be 25–27 °C for *S. mansoni* and *S. haematobium*, with water temperatures less than 10 °C and greater than 40 °C resulting in significantly more cercariae dying during penetration (Ghandour, 1976). Water temperatures of 24–28 °C also result in maximum worm burden (Purnell, 1966; Lwambo *et al.*, 1987), however at water temperatures typically encountered in endemic regions, there is no real impact on infectivity or subsequent worm burden, and attachment is more dependent on the age of cercariae rather than water temperature (Lawson and Wilson, 1980; Lee *et al.*, 2013). Over 20% of initial glycogen stores is used during skin penetration (Lawson and Wilson, 1980).

2.1.4.5 Transformation to schistosomula

Transformation to schistosomula is marked by the shedding of the tail and glycocalyx. The majority (60–88%) of cercariae shed their tails after penetrating the host, bring the tail with them into the epidermis (Whitfield *et al.*, 2003b; Haas and Haeberlein, 2009). Here the glycocalyx is either partially or totally shed (Stirewalt, 1974). Schistosomula initially travel parallel to the outermost layer of the skin, before travelling deeper into the epidermis, guided by chemical gradients of glucose and arginine and the lack of light (Grabe and Haas, 2004; He *et al.*, 2005). After a few hours (*S. japonicum*) or

a few days (*S. mansoni* and *S. haematobium*) they enter the dermis and then leave the skin via a blood or lymphatic vessel and are transported in the circulatory system through the lungs and heart before entering the liver where they mature into worms and begin mate finding, completing the life cycle (He *et al.*, 2002; Nation *et al.*, 2020). The prepatent period in humans, the time between skin penetration and release of eggs, is 24–63 days, depending on the species (Burden and Ubelaker, 1981; He *et al.*, 2002). It is unclear why *S. japonicum* schistosomula are able to migrate through the body much faster than the other human schistosomes, but it has been suggested that they may posses more potent enzymes. This could also explain why they are able to infect such a broad range of mammalian hosts (He *et al.*, 2002).

2.2 Schistosomiasis

2.2.1 Distribution

Schistosomiasis is a global disease with transmission reported in 78 countries, although it is estimated that 80–90% of infections occur in sub-Saharan Africa (Chitsulo *et al.*, 2000). Transmission is highly localised and is dependent on a number of factors: the presence of the intermediate host, water contact behaviour, inadequate containment of faeces and urine, and suitable water conditions to enable the survival of miracidia and cercariae (see Section 2.2.2). Endemicity may also change with migration and man-made interventions such as damming and irrigation (Southgate, 1997; Steinmann *et al.*, 2006; Wang *et al.*, 2013; Boissier *et al.*, 2016). *S. mansoni* is thought to have been imported to Latin America and the Caribbean islands via the trans-Atlantic slave trade and the prevalence of schistosomiasis in Egypt increased dramatically following the introduction of irrigation canals, with an estimated 70% of males infected with *S. haematobium* in the 1920's (Abdel-Wahab *et al.*, 1979; Lammie *et al.*, 2007; Bella *et al.*, 2018).
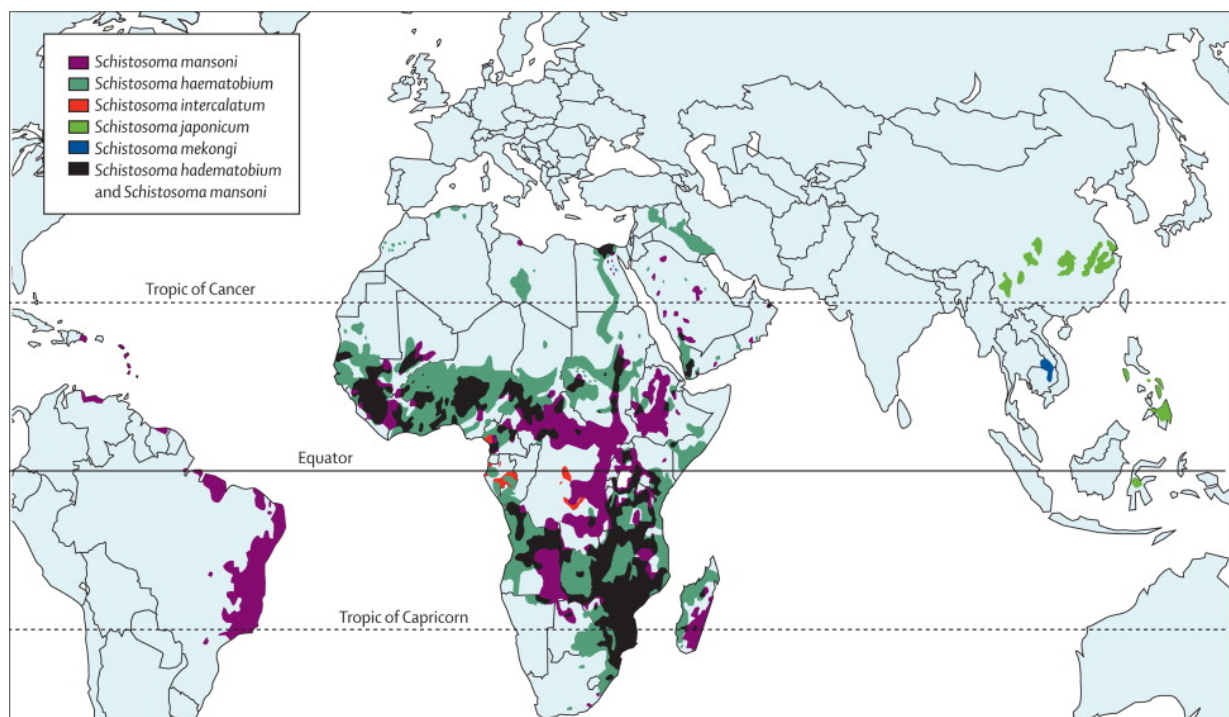
Figure 2.8 Geographical ranges of human schistosome species. From Ferrari and Moreira (2011) with data from Chitsulo *et al.* (2000) and Gryseels *et al.* (2006), reproduced with permission.

Each schistosome species has a specific intermediate snail host and the geographical distribution of schistosomiasis is therefore dependent on the habitat ranges of the snails (Figure 2.8). In addition to the three main human species which are present in Africa and the Middle East (*S. haematobium* and *S. mansoni*), Latin America (*S. mansoni*), and South-East Asia (*S. japonicum*), three further human species are responsible for localised transmission in west and central Africa (*S. guineensis* and *S. intercalatum*) and the Mekong River basin (*S. mekongi*) (Colley *et al.*, 2014).

As schistosomes are dioecious there is also the potential for interbreeding between species where geographical ranges overlap (Leger and Webster, 2017). Hybridisation between human and animal schistosomes (of which there are over 20 known species), and the implications for control measures, is of particular concern. Hybrid species may be able to infect a greater range of host species, which in turn may alter the geographic distribution of schistosomiasis (King *et al.*, 2015; Leger and Webster, 2017; Kincaid-Smith *et al.*, 2017). *S. haematobium–S. bovis* hybrids have caused outbreaks of urogenital schistosomiasis in Corsica, France, since 2013, and a similar hybrid may have been responsible for an epidemic in Portugal in the early 20th century (Boissier *et al.*, 2016; Rothe *et al.*, 2021).

In the near future, rising water temperatures and changes to rainfall patterns resulting from man-made climate change may alter the distribution of schistosomiasis. Whilst there is no evidence to suggest the outbreaks in Corsica were a result of climate change, it was previously considered to be outside to the geographical range of schistosomiasis transmission, due to near-freezing water temperatures in winter (Leo *et al.*, 2020). Indeed models predict that large areas of southern Europe will become habitable for intermediate snails hosts by the middle of the 21st century (Yang and Bergquist, 2018; Leo *et al.*, 2020). Climate change is a complex problem and predicting the impact on schistosomiasis distribution equally so (Stensgaard *et al.*, 2019). However it is anticipated that rising temperatures will expand the potential transmission range of schistosomiasis into cooler regions, whilst reducing the current range of some snail habits that will become prone to droughts (Blum and Hotez, 2018; Yang and Bergquist, 2018; Adekiya *et al.*, 2020; Leo *et al.*, 2020). More extreme flooding events may also lead to snail dispersal resulting in epidemic outbreaks in endemic regions (Yang and Bergquist, 2018). Climate change is also expected to indirectly affect schistosomiasis transmission as a result of increased poverty, lack of access to WASH, increased migration, dam development, and agriculture expansion (Wang *et al.*, 2013; Yang and Bergquist, 2018; Leo *et al.*, 2020).

2.2.2 Transmission risk factors

The probability of contracting schistosomiasis is dependent on a number of interlinked factors that fall into three categories: parasite-, snail-, and human-related risk factors (Table 2.2, Walz *et al.*, 2015). Parasite- and snail-related factors are species specific and primarily related to environmental conditions such as temperature, water velocity, and vegetation. Of the human-related risk factors water contact behaviour is the most important.

In terms of domestic or recreational activity, bathing, swimming, and laundry are considered to be the most high risk water contact behaviours for schistosomiasis transmission due to the frequency and duration of water contact and the area of the body submerged. Many studies have demonstrated a positive correlation between these activities and schistosome infection (Kloos *et al.*, 1983; Sama *et al.*, 2007; M'Bra *et al.*, 2018). Furthermore, cercariae are able to adapt their circadian shedding pattern

Table 2.2 Some of the main parasite-, snail- and human-related risk factors that affect schistosomiasis transmission. Adapted from Walz *et al.* (2015).

Parasite	Effect on schistosomiasis transmission	References
Temperature	Affects prepatent period, survival, activity, and infectivity of miracidia and cercariae. Cercariae have adapted their shedding patterns so that peak emergence is when it is hottest, to coincide with peak water activity of their host.	Stirewalt, 1954; Jamieson *et al.*, 2017; Lawson and Wilson, 1980; Ghandour, 1976; Combes *et al.*, 1994
Water velocity	Concentration of cercariae is highest in stagnant water, flowing water can sweep cercariae away from snail habitats and deposit them downstream. Very slow moving water (∼0.1 m/s) leads to widespread dissemination of miracidia, increasing the opportunities for host finding.	Rowan, 1958; Upatham, 1973
Predators	Some fish and invertebrates feed on miracidia and cercariae, reducing the parasite population	Jordan and Webbe, 1982

Snail	Effect on schistosomiasis transmission	References
Temperature	Affects survival, fecundity, and rate of reproduction.	Abdel Malek, 1958; Shiff, 1964
Water velocity	Velocities over 0.3 m/s will dislodge snails.	WHO, 1957; Sturrock, 2001
Vegetation	Snails require certain types of vegetation for food and depositing egg masses. Vegetation also increases the dissolved oxygen in water which is linked to reproduction rates.	Abdel Malek, 1958
Water depth	Snails are rarely found in water depths over 1.5 m, reducing the risk of transmission of schistosomiasis at these depths.	WHO, 1957
Water quality	Snails and eggs are sensitive to turbidity, mineral content, and pH, as well as chemical pollutants.	WHO, 1957; Sturrock, 2001
Rainfall	Cercarial concentrations are proportional to the number of snails which increases in the rainy seasons. Heavy rainfall may increase water velocity, resulting in the dispersal of snails and the creation of new habitats. It also increases contamination of water bodies with human excrement.	Chandiwana, 1986; Mohoho *et al.*, 1997

Human	Effect on schistosomiasis transmission	References
Water contact	Skin exposure to water containing cercariae is required for human infection. Increased frequency, duration, and area of body exposed to water increase the risk of infection.	Kloos *et al.*, 1983; Sama *et al.*, 2007; M'Bra *et al.*, 2018
Hygiene	Contamination of water with faeces or urine releases eggs into the environment.	Huang and Manderson, 1992
Gender	Relationship between gender and risk of schistosome infection is culturally variable and a determinant of water contact activities.	Huang and Manderson, 1992; Gazzinelli *et al.*, 2001; Okanla *et al.*, 2003; Gyuse *et al.*, 2010
Age	Children and adolescents have higher risk of infection due to water contact behaviour and low level of immunity, which increases with exposure.	Woolhouse, 1998; Mutapi *et al.*, 2008; Colley *et al.*, 2014
Occupation	Water related occupations such as fishing and farming increases water contact.	Farooq *et al.*, 1996; Dalton and Pole, 1978; Huang and Manderson, 1992
Religion	Some religious or cultural norms related to water contact (e.g. ritual cleansing) can increase contamination and exposure.	Jordan and Webbe, 1982
Socioeconomics	Availability of WASH infrastructure and ability to cope with the disease is related to socioeconomic status.	Lima e Costa *et al.*, 1987

so that it coincides with peak water contact activity of the definitive hosts (Théron, 1984; Combes *et al.*, 1994; Aoki *et al.*, 2003; Mouahid *et al.*, 2019). Emergence of human infecting species tends to peak between 1100–1300 when it is hottest and there is likely to be an increase in activities such as bathing and laundry, whilst animal species tend to shed more at dawn or dusk (Mouahid *et al.*, 1991; Combes *et al.*, 1994). *S. rodhaini*, which infects wild rodents, is the only nocturnal schistosome species, although some strains of *S. mansoni* which have limited human contact have adapted their shedding patterns to late evening so that is coincide with the nocturnal activity of rats, a second definitive host for *S. mansoni* in some regions (Théron, 1984; Théron and Combes, 1988; Mouahid *et al.*, 2019).

Collecting water is generally considered to be a low risk activity as only a small area of the body is exposed and it is often carried out in the morning, when cercarial concentrations are low (Kloos *et al.*, 1983; Walz *et al.*, 2015). However children may become more submerged when collecting clearer water which is located further from the shore and commercial water collection may also take place throughout the day (Trienekens *et al.*, 2020). Furthermore, the time of peak emergence can vary and does not necessarily indicate time of peak concentration of cercariae (Wolmarans *et al.*, 2002). A study in Puerto Rico found the concentration of *S. mansoni* cercariae in rivers and streams peaked between 1100 and 1300, whereas in non-flowing ponds the numbers of cercariae would accumulate throughout the day before peaking at approximately 1600 (Rowan, 1958). Given the lifespan of cercariae, it should be noted that no time of the day can be considered completely "safe" in endemic areas (Jones and Brady, 1947b) and whilst the act of collecting water may be considered low risk, it is still possible to contract schistosomiasis if the water is subsequently used for laundry or bathing without being treated.

Most researchers agree that males are at higher risk of schistosomiasis infection than females, due to their higher engagement in activities such as swimming, fishing, farming, and watering livestock, and there are a number of studies that support this (El-Khoby *et al.*, 2000; Sama *et al.*, 2007; Liao *et al.*, 2011; Geleta *et al.*, 2015; M'Bra *et al.*, 2018). However this is not universal, as demonstrated by studies in Nigeria, Ghana, Brazil, and Uganda, and is dependent on cultural issues that govern water contact behaviour (Huang and Manderson, 1992; Gazzinelli *et al.*, 2001; Okanla *et al.*, 2003; Gyuse *et al.*, 2010; Trienekens *et al.*, 2020). An observational study in Zimbabwe found that the frequency of water contact activities was four times higher for women than men and the average duration of contact was also longer. This resulted in prevalence of 50% for women and 30% for men for *S. haematobium* infection, and 53% for women and 38% for men for *S. mansoni* infection (Husting, 1983). Religion can play a role in governing water contact behaviours between genders (Kloos *et al.*, 1983) and may also impact other behaviours. Ablution rituals which are required before prayer and may occur five times a day in Muslim communities and have been linked to an increase in prevalence of schistosomiasis (Jordan and Webbe, 1982). Anal cleansing in particular has been shown to release 12 times as many eggs into the environment, when compared to one stool being deposited into the water (Sow *et al.*, 2008).

Children and adolescents are considered to be at the highest risk of infection due to increased water contact activity and lack of acquired immunity (Woolhouse, 1998; Mutapi *et al.*, 2008; Colley *et al.*, 2014). Initial infection normally occurs at a young age (pre-school) and has been linked to mothers bringing infant children to water sites during domestic activities (Dalton, 1976; Garba *et al.*, 2010; Seto *et al.*, 2012; Poole *et al.*, 2014). Infection generally increases in prevalence and intensity throughout childhood before peaking in adolescence and then declining into adulthood (King *et al.*, 2004; Mutapi

et al., 2008; Walz *et al.*, 2015; Turner *et al.*, 2020; Toor *et al.*, 2020). However, age-infection profiles are dependent on local conditions (Woolhouse, 1998; Silva *et al.*, 2019) and high prevalence can persist into adulthood amid some sub-populations, most notably in communities that are economically reliant on fishing and farming, with these two occupations unsurprisingly linked to high prevalence among adults (Farooq *et al.*, 1966; Huang and Manderson, 1992; Pullanikkatil *et al.*, 2014). The decline in adulthood has been linked to immunity from childhood infections, skin thickness, and a reduction in water contact behaviour (Dalton and Pole, 1978; Fulford *et al.*, 1998; Kabatereine *et al.*, 1999).

2.2.3   Morbidity

Morbidity arising from schistosomiasis is mainly caused by the hosts' immune response to eggs that become trapped in the body, rather than the adult worms (McManus *et al.*, 2018). Acute schistosomiasis, also known as Katayama syndrome, most commonly occurs in adults who have not been exposed to schistosome antigens at a young age, such as travellers or immigrants to endemic regions (Colley *et al.*, 2014). Initial skin penetration can cause an itchy rash known as cercarial dermatitis or "swimmer's itch", caused by the body's immune response to cercariae. The rash normally appears within 24 hours but is short lived and may go unnoticed (Meltzer *et al.*, 2006). Cercarial dermatitis is also caused by mammalian and avian schistosome cercariae, which are able to penetrate human skin even though they are unable to develop into adult worms (Hoeffler, 1974). The next phase of symptoms occurs 2–12 weeks after cercarial exposure and are thought to be caused by an allergic reaction to antigens released by migrating schistosomula and early egg development, although Katayama syndrome has been shown to occur in the absence of eggs (Ross *et al.*, 2007; Langenberg *et al.*, 2019). Clinical manifestations are non-specific and may include sudden fever, coughing, headaches, muscle pain, abdominal tenderness, malaise, and fatigue. As a result, acute schistosomiasis is often misdiagnosed, particularly in non-endemic countries where there may be limited knowledge of the disease among health practitioners (Ross *et al.*, 2007; Rochat *et al.*, 2015).

If treatment is not provided, over time the disease will progress to chronic schistosomiasis, the symptoms of which vary depending on the species and where the eggs are trapped. The eggs of *S. mansoni* and the other intestinal schistosomes generally become trapped in the liver or spleen, whereas the eggs of *S. haematobium* travel to the bladder or urogenital system. Antigens released by eggs cause a granulomatous immune response in the host. These granuloma contain proteolytic enzymes which prevent tissue necrosis, but also induce chronic inflammation and lesions (Colley *et al.*, 2014).

Intestinal schistosomiasis presents as abdominal pain, diarrhoea, and rectal bleeding. The granulomatous response to eggs can cause enlargement of the liver or spleen which may increase the pressure in the portal vein (portal hypertension) and can lead to a buildup of fluid in the abdomen (ascites), which is evidenced by a distended abdomen (Doehring, 1988). Portal hypertension can result in eggs becoming dislodged and migrating to the lungs where they can cause pulmonary hypertension (high blood pressure in the blood vessels of the lungs) and lung disease (Schwartz, 2002; Graham *et al.*, 2010). The defining symptom of urogenital schistosomiasis is bloody urine, along with pain or burning during urination. In severe cases, inflammation of the urinary tract can cause obstructions which may lead to bacterial infection and/or kidney failure and lesions in the bladder may give rise to bladder cancer (Ferguson, 1911; Malik *et al.*, 1975; Silva *et al.*, 2013).

Lesions in the reproductive organs causes an under-diagnosed condition called genital schistosomiasis (Christinet *et al.*, 2016; Kayuni *et al.*, 2019). Genital schistosomiasis is primarily caused by *S. haematobium* eggs but it can also be caused by the eggs of intestinal species (Kjetland *et al.*, 2012). Despite the clinical manifestations in females being first recorded in 1899, it is one of the most understudied areas of schistosomiasis research (Christinet *et al.*, 2016). The symptoms of male (MGS) and female genital schistosomiasis (FGS) are non-specific and include genital discomfort and pain (particularly during intercourse), stress incontinence, erectile discomfort and dysfunction, and infertility. FGS and MGS have also both been linked to increased susceptibility to HIV infection and to cervical and prostate cancer, respectively (Kjetland *et al.*, 2006; Bacelar *et al.*, 2007). Proper diagnosis requires invasive procedures by trained health care workers and as a result it is often misdiagnosed as a sexually transmitted disease. The social stigma associated with such diseases can lead to marital discord and mental distress among sufferers of FGS and MGS (Christinet *et al.*, 2016).

Although more rare than intestinal and urogenital disease, eggs of all species can become trapped in the central nervous system leading to neuroschistosomiasis. *S. japonicum* eggs account for 60% of all brain infections due to the small size of the eggs and 2–4% of *S. japonicum* infections are thought to involve cerebral disease (Watt *et al.*, 1986; Verjee, 2019). *S. haematobium* and *S. mansoni* eggs are more likely to become trapped in the spinal cord (Verjee, 2019). Symptoms of neuroschistosomiasis include seizures, delirium, headaches, nausea and vomiting, visual field impairment, paraplegia, loss of bowel and bladder control, and sensory disturbances (Carod-Artal, 2008; Ferrari and Moreira, 2011; Verjee, 2019).

Non-specific disabling morbidity caused by all schistosome species includes chronic abdominal pain, diarrhoea, anaemia, malnutrition, and impaired growth and cognitive development, particularly in children (Parraga *et al.*, 1996; Nokes *et al.*, 1999; McGarvey, 2000; Friedman *et al.*, 2005; King *et al.*, 2005; Ezeamama *et al.*, 2018). Whilst these symptoms are often overlooked, evidence suggests they are far more prevalent than symptoms of severe schistosomiasis and some researchers believe they may represent the greatest burden of chronic schistosomiasis (King *et al.*, 2005; King and Dangerfield-Cha, 2008).

2.2.4 Burden of disease

It is difficult to estimate the number of individuals currently infected with schistosomes because transmission is highly localised, so survey data from one community may not reflect transmission in the wider areas. According to the latest Global Burden of Disease (GBD) study the number of active infections in 2016 was approximately 190 million (GBD 2016 Collaborators, 2017), whilst the WHO data state the total number of people requiring preventive chemotherapy (PC) in 2020 was 236 million people, in 51 countries (WHO, 2020a). However, due to the insensitivity of field diagnostic techniques which often miss low-intensity infections, some researchers estimate the actual prevalence is 2–3 times higher (Vlas and Gryseels, 1992; Wilson *et al.*, 2006; King, 2010; King and Galvani, 2018). In addition to active infections, approximately 210 million people are suffering with symptoms from past infections that persist even after treatment to remove the worms (Giboda and Bergquist, 1999; Kjetland *et al.*, 2008; Colley *et al.*, 2014), therefore the number of people living with disease caused by schistosomiasis may be in excess of 500 million, of which approximately 20 million people are thought to suffer from severe symptoms (Chitsulo *et al.*, 2000; Steinmann *et al.*, 2006).

It is well known that severe cases of schistosomiasis can lead to death, but mortality estimates vary considerably. In 2002 the WHO Expert Committee estimated that in sub-Saharan Africa alone, 200,000 people die annually from schistosomiasis, stating that historical evidence from Brazil showed that 1% of people will die from the disease if there is no treatment (WHO, 2002). Similarly, Werf *et al.* (2003) suggest 150,000 and 130,000 people die each year from schistosomiasis related kidney disease (*S. haematobium*) and haematemesis (*S. mansoni*), respectively. There have been many advances in control campaigns since 2002 and the GBD 2016 study states annual deaths caused by schistosomiasis are approximately 10,000 people each year (GBD 2016 Collaborators, 2017), however many researchers still refer to an estimated 200,000 annual deaths. Nevertheless, schistosomiasis is still regarded as a disabling disease rather than a deadly disease, and the major burden worldwide is as a result of chronic morbidity.

Disease burden is quantified in disability-adjusted life years (DALYs), i.e. the number of years lost to ill-health, disability, or early death. The latest GBD study estimates schistosomiasis leads to 1.9 million DALYs, making it the second most disabling NTD after soil-transmitted helminthiases (GBD 2016 Collaborators, 2017). Even so, many researchers believe this is a underestimate of the true burden of the disease, as it does not quantify the compounding effects of co-infections (e.g. with soil-transmited helminthiases (STH) or human immunodeficiency virus (HIV)) and the disability weighting for schistosomiasis overlooks the "subtle" but widespread morbidities caused by low-intensity infections such as malnutrition and cognitive impairment (Engels and Savioli, 2006; King *et al.*, 2005; King and Dangerfield-Cha, 2008; King and Galvani, 2018). The GBD study did also not include the effects of genital schistosomiasis in its calculations, the effect of which have been under-recognised for decades (Goldberg *et al.*, 2018). King (2010) suggests the true burden may be as high as 56 million DALYs, based on a higher prevalence estimate and disability weighting. It should also be noted that DALY calculations do not reflect non-clinical burdens of the disease, such as the impacts on school attendance and economic productivity, and the feedback loop that exists between disease and poverty (Engels and Savioli, 2006; King, 2010; Miguel and Kremer, 2004; Baird *et al.*, 2016).

2.3 Schistosomiasis control

2.3.1 History of schistosomiasis control

Schistosomiasis is an ancient disease with the earliest archaeological evidence of humans infected with schistosomes dated 6000–6500 years before present (Figure 2.9) and several descriptions of symptoms characteristic of schistosomiasis in ancient Egyptian, Assyrian, and Chinese texts (Anastasiou *et al.*, 2014; Bella *et al.*, 2018; Zhou *et al.*, 2021). The first clinical description of schistosomiasis (then called Katayama disease) was recorded in 1847 by a practitioner of Chinese medicine, Yoshinao Fuhii, in his memoirs from Katayama, Japan (Tanaka and Tsuji, 1997). The disease was linked to water contact but the parasite was not discovered until four years later in 1851 in Cairo, Egypt, by Bilharz who named his discovery *Distomum haematobium* (Bilharz, 1853). Weinland renamed the genus *Schistosoma*, meaning "split-body", in 1858 after the morphology of the male worms (Bella *et al.*, 2018). In 1904 *S. japonicum* was first discovered in a cat in Yamanashi, Japan, by Katsurada, and four days later in a man in Katayama by Fujinami (Ishii *et al.*, 2003). The life cycle of *S. japonicum* was the first to be described in full by Miyairi and Suzuki (1914), who discovered that aquatic snails acted as the
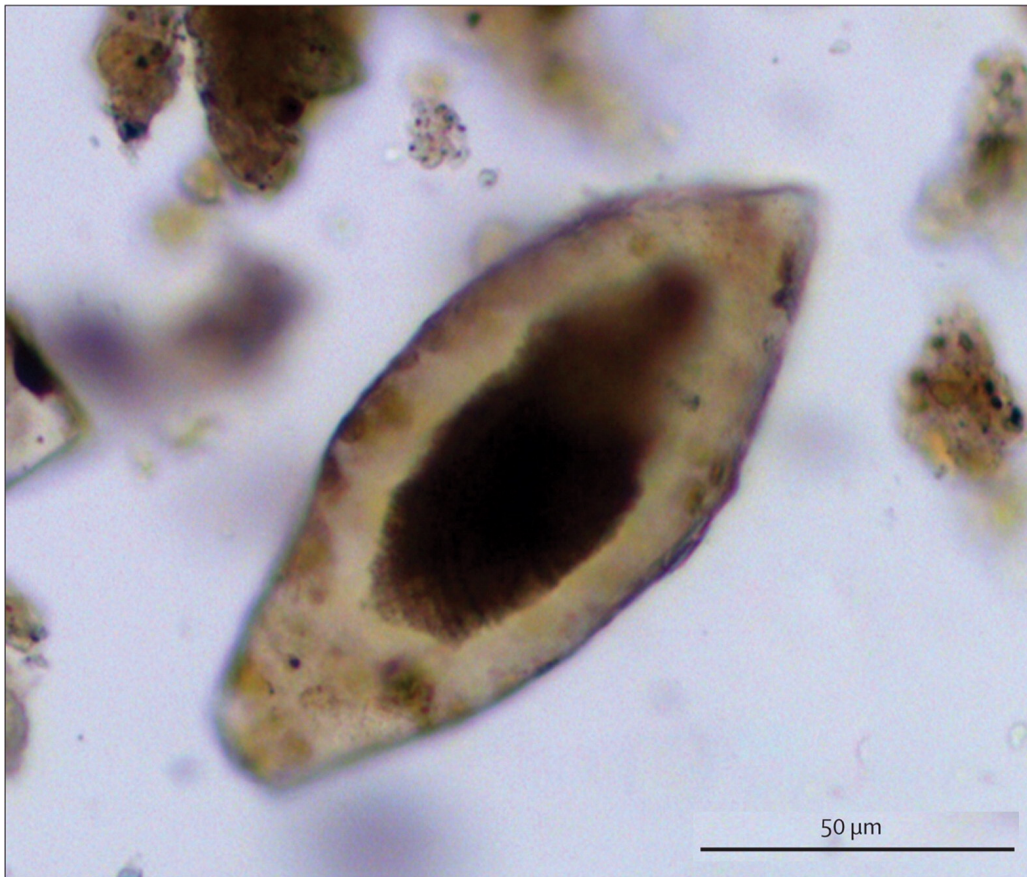
Figure 2.9 A terminal spined *Schistosoma haematobium* or *Schistosoma intercalatum* egg recovered from the pelvic sediment of human skeletal remains excavated in norther Syria and dated 6500–6000 before present. From Anastasiou *et al.* (2014), with permission.

intermediate host. Shortly afterwards, Leiper (1915) was the first to differentiate between the life cycles of *S. haematobium* and *S. mansoni* in Egypt.

Without full knowledge of the life cycle, early policies for preventing schistosomiasis primarily focused on avoiding water contact and hygiene education to prevent contamination of water sources. However, without proper funding for improvements in WASH infrastructure, these campaigns were not particularly effective (Sandbach, 1976; Jordan, 2000). Following the discovery of the intermediate host, research shifted towards technical solutions to control snails and eliminate the parasites, which offered the possibility of a quick and affordable solution to schistosomiasis control.

Leiper (1915) suggested the most effective method of control was to eliminate snails through periodic drying of irrigation canals, mollusciciding, and canal clearances to remove snail habitats. He also recommended treating water by storing, heating, or using sodium bisulphate or chlorine to kill cercariae. These measures were adopted in the British War Office memorandum on tropical diseases in 1919 to combat schistosomiasis infections in troops stationed Africa and China, along with a new treatment, tartar emetic, which was first tested on patients in 1918 (Christopherson, 1918; Royal Army Medical Corps, 1919). This relatively cheap treatment relieved symptoms and stopped egg output, but could have dangerous side effects, so it had to be administered slowly over a period of 2–3 weeks (Jordan, 2000). Nevertheless it was effective and was widely used as treatment for schistosomiasis until the development of praziquantel in the late 1970's (Katz *et al.*, 1979). Tartar emetic was used in early PC campaigns in Egypt in the 1920's, which successfully reduced morbidity in the population but the

treatment itself also caused thousands of deaths (Khalil, 1936; Jordan, 2000).

The dual discoveries of the intermediate host and an affordable treatment (tartar emetic) led many researchers to believe schistosomiasis could be quickly controlled and even eliminated in Egypt within a few years, but by the 1930's it became clear that this would not be the case (Jordan, 2000). In fact infections actually increased throughout the early 20th century as a result of population growth, migration, and extensive water infrastructure projects that expanded the habitat range of the intermediate host snails (Sturrock, 2001a). After World War II and the development of cheaper and more effective molluscicides (such as niclosamide which is still used today) snail control gained further prominence and remained the main policy for schistosomiasis control until the 1980's. During this time Japan successfully eliminated schistosomiasis through sustained campaigns of epidemiological surveys, snail control, and environmental management (WHO, 1993; Tanaka and Tsuji, 1997; Bergquist and Adugna, 2017). This was likely aided by the highly localised nature of schistosomiasis transmission in Japan and rapid economic development in the second half of the 20th century that improved living conditions and access to WASH and health care. The last human infection was recorded in 1977 and elimination was formally declared in 1996 (Kajihara and Hirayama, 2011).

The anthelmintic drug praziquantel was developed in the late 1970's, and following successful clinical trials on all three main species which demonstrated it was both safe and very effective (Katz *et al.*, 1979; McMahon and Kolstrup, 1979; Santos *et al.*, 1979), it has been widely used for treatment and control of schistosomiasis since the 1980's. Following the success of PC programmes with praziquantel in Egypt, Brazil, Morocco, and China, in 2001 the WHO endorsed regular treatment of high-risk groups as the best means of reducing mortality and morbidity from schistosomiasis in endemic regions, urging member states to commit to regular administration of praziquantel to at least 75% of school aged children by 2010 (World Health Assembly Resolution 54.19). Whilst this target was not achieved, PC has been the primary component of almost all schistosomiasis control programmes ever since and is still the main control method endorsed by the WHO.

Vaccination has historically been a very cost effective method of controlling infectious diseases and there have been hundreds of potential vaccine candidates for schistosomiasis (Hotez *et al.*, 2019a; McManus *et al.*, 2020; Molehin, 2020). Within the last decade four have advanced to clinical trials, however the first vaccine to reach Phase 3 human trials in Africa was found to offer no reduction in worm burden or egg output (Molehin, 2020; Panzner *et al.*, 2021).

Countries that have been successful at interrupting or reducing transmission, such as Japan, China, Iran, Morocco, Tunisia, St Lucia, and other Caribbean islands, have implemented integrated campaigns of chemotherapy, snail control, and improving standards of living and healthcare (Rollinson *et al.*, 2013). Since the discovery of the life cycle, many researchers and organisations including the WHO have consistently advocated for this integrated approach to schistosomiasis control to be widely adopted (Leiper, 1915; Farooq, 1964; Iarotski and Davis, 1981; Liese, 1986; WHO, 1993; Singer and Castro, 2007; Holveck *et al.*, 2007; Utzinger *et al.*, 2009) however with little formal guidance on how to implement such programs most national programmes in sub-Saharan Africa are still struggling to achieve this today. The four arms of an integrated approach to schistosomiasis control (Figure 2.10) are discussed in more detail in the following sections.
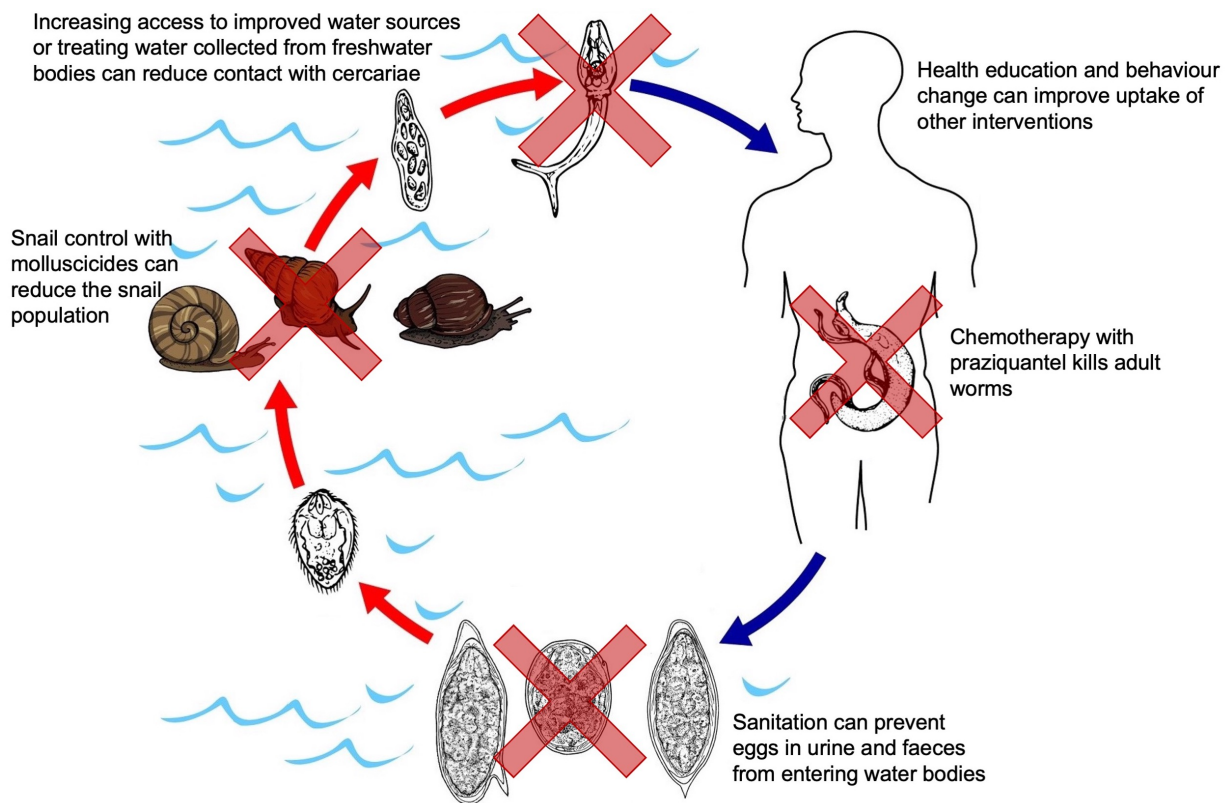
Figure 2.10 Interventions that can interrupt the schistosome life cycle include chemotherapy, snail control, water, sanitation, and hygiene, and behaviour change. Adapted from CDC (2012).

2.3.2 Preventive chemotherapy

Praziquantel can be safely administered to anyone over the age of four at a standard oral dose of $40\,\mathrm{mg/kg}$ with trials for paediatric formulations in progress (Reinhard-Rupp and Klohe, 2017; Webb *et al.*, 2021). The risk of side-effects, including abdominal pain, headaches, and dizziness, increases with intensity of infection, however they are usually short lived and praziquantel is generally well-tolerated at the recommended dose (Colley *et al.*, 2014). The drug kills adult worms and is effective against all human schistosomes with cure rates of approximately 60–90% and an optimal egg reduction rate of 90% (WHO, 2013; Inobaya *et al.*, 2014). It is not effective against juvenile worms and 100% cure rates are rarely, if ever, achieved in endemic areas where people are likely to be infected with different developmental stages of the parasite (Doenhoff *et al.*, 2008). Very low cure rates have been observed in some studies, these have been linked to high initial worm burdens, intensity of transmission, high reinfection rates, host age, and a reduction in efficacy of praziquantel following multiple rounds of treatment (Utzinger *et al.*, 2000; Gryseels *et al.*, 2001; Crellen *et al.*, 2016). In general mass drug administration (MDA) of praziquantel has been very effective at reducing prevalence and intensity of infection in many regions, which in turn reduces the risk of developing chronic schistosomiasis and associated morbidity (Kheir, 2000; Guidi *et al.*, 2010; Sarvel *et al.*, 2011). However, hotspots remain and are not reducing as expected, and other interventions are therefore required.

Aided by funding from international organisations and drug donations by the pharmaceutical company Merck, the WHO distributes praziquantel to at-risk populations via preventive chemotherapy campaigns, in which all participants are regularly treated, irrespective of infection status. These campaigns primarily target school-age children (5–14 years) as prevalence is highest among these ages

groups and school settings enable efficient administration (The Partnership for Child Development, 1999; Guyatt *et al.*, 2001; Kabatereine *et al.*, 2007). The WHO currently recommends annual PC for school-aged children (SAC) and at-risk adults in high-prevalence communities (>50% prevalence in SAC), reducing to every other year in communities where prevalence is moderate (10–50% in SAC). In low-prevalence communities (<10% in SAC) the WHO recommends two rounds of treatment for children during primary schooling (WHO, 2006). Whilst administration in schools has undoubtedly been effective at reducing helminth infections, and over 85 million children were treated in 2019 (WHO, 2020a), these campaigns do not reach children who do not attend school either because they are too young or because of inherent education inequalities such as gender (Rollinson *et al.*, 2013; Bustinduy *et al.*, 2016). In 2001 the WHO aimed to reach 75% of SAC a year with PC by 2010, but by 2019 it was estimated that only 67% had been reached (WHO, 2020b), in spite of the extensive praziquantel donation programme which has been running for over 10 years. Globally it is estimated that in 2019 only 44% of the at-risk population had access to treatment, irrespective of age (WHO, 2020a).

Treatment coverage is critical to the success of control programmes that rely on PC as a small number of heavily infected individuals can produce enough eggs to maintain transmission in an entire community (King *et al.*, 1991; Talaat *et al.*, 1999). A fundamental challenge, particularly in low prevalence communities, is that many people do not know they are infected and do not perceive schistosomiasis to be a health problem (Mwanga *et al.*, 2020). Misinformation or fear of side affects can also cause people to miss treatments (Omedo *et al.*, 2012). Coverage can be improved by health campaigns and community involvement (Tallo *et al.*, 2008), however even if 100% coverage were possible, transmission is unlikely to be interrupted by PC alone because praziquantel is not 100% effective and it cannot prevent reinfection (Doenhoff *et al.*, 2009). A reservoir of infection therefore always remains and once PC is ceased prevalence can return to baseline levels within a few years if no other control measures are introduced (Guidi *et al.*, 2010). Furthermore the reliance on a single form of treatment, almost all of which is donated by a private company, leaves PC campaigns vulnerable to both supply chain issues and drug resistance. Resistance to praziquantel has been induced *in vitro* in schistosomes from both Egypt and Senegal (Fallon *et al.*, 1995; Ismail *et al.*, 1996; Ismail *et al.*, 1999) and there are several reports of repeated treatment failure in individuals, primarily in returning travellers from non-endemic areas, however there is currently no definitive evidence of widespread resistance occurring in the field, in spite of extensive administration in endemic regions (Whitty *et al.*, 2000; Silva *et al.*, 2005; Alonso *et al.*, 2006; Wang *et al.*, 2012). Nevertheless, as current control measures are centred around PC the threat of emerging resistance remains.

2.3.3 Snail control

Snail control, primarily through the application of chemical molluscicides, played an important role in the elimination of schistosomiasis in Japan, and is a key component of the control programme in China (Tanaka and Tsuji, 1997; Rollinson *et al.*, 2013). It reduces the number of intermediate snail hosts and therefore reduces the number of cercariae in the water. Niclosamide has been the main molluscicide used since the 1960s, it is effective against all development stages of the snail life cycle and has the added benefit of being toxic to schistosome cercariae (Lardans and Dissous, 1998). It has low toxicity to humans and livestock in low doses and can simultaneous be used to control the vectors of other trematodes that infect domestic animals and people (King and Bertsch, 2015). However, total eradication of snails is unlikely and as snails are hermaphrodites they are able to repopulate very

quickly, therefore regular application is required to keep numbers low (Inobaya *et al.*, 2014). This incurs considerable labour costs which are estimated to be responsible for 60% of the total cost of snail control campaigns (King and Bertsch, 2015; Coelho and Caldeira, 2016). Furthermore niclosamide is known to be toxic to other non-target organisms and long term and large scale use in China has resulted in near ubiquitous pollution of the water and soil in schistosomiasis endemic regions (Zhu *et al.*, 2020; Huang *et al.*, 2013). Niclosamide has also been shown to bioaccumulate in the edible tissues of fish, although current data suggests the levels are not harmful to humans (Liu *et al.*, 2018). Mollusciciding is therefore best used in a targeted manner, to enhance PC campaigns. MDA is most effective when the risk of reinfection is low, therefore snail control and PC campaigns should be coordinated such that snail populations are reduced prior to drug administration (Sturrock, 1995; Rollinson *et al.*, 2013). Snail control can also be particularly useful in areas that have achieved low tranmission rates and are vulnerable to disease resurgence through migration from other endemic regions (Fenwick *et al.*, 2006).

Other non-chemical forms of snail control include the use of plant based compounds such as endod (*Phytolacca dodecandra*, also known as soapberry), clearing vegetation around water bodies to reduce snail habitats, and biological control through the introduction of competitive snail species or predators; although these methods may also have unintended ecological consequences (Slootweg *et al.*, 1994; Sturrock, 1995; Boelee and Laamrani, 2004; Abebe *et al.*, 2005; Qiu *et al.*, 2020).

2.3.4 Water, sanitation, and hygiene

Improving access to water, sanitation, and hygiene is one of the cornerstones of disease prevention and poverty reduction and it is estimated that 3.9% of the global disease burden and 2.8% of all deaths are attributable to inadequate WASH access in low- and middle-income countries (Prüss-Ustün *et al.*, 2019). "Water" refers to domestic water supplies primarily for drinking but also for other domestic activities such as cooking, bathing, and laundry, with water-contact activities being particularly important for preventing transmission of schistosomiasis. "Sanitation" refers to the management and disposal of human excreta, and "hygiene" refers to the practices required to promote health and prevent the spread of diseases including regular handwashing with soap. The WHO and United Nations Children's Fund (UNICEF) Joint Monitoring Program (JMP) monitors global water and sanitation coverage and has set out a hierarchy for WASH resources (Table 2.3). In 2020 26% of the global population did not have access to a safely managed water supply, including 122 million people who still rely on surface water for drinking water, and 46% did not have access to safely managed sanitation services, with 496 million people practising open defecation (JMP, 2021). It should be noted that this data is for drinking water and does not include the number of people who use unimproved sources for other domestic activities.

There is considerable overlap between regions where WASH access is insufficient and areas that are endemic to schistosomiasis, and it is estimated that 43–82% of the overall burden of schistosomiasis is attributable to inadequate WASH access (Prüss-Üstün *et al.*, 2016; Prüss-Ustün *et al.*, 2019). As each of the three pillars of WASH target different developmental stages of the schistosome life cycle they have been reviewed separately.

Table 2.3 Joint Monitoring Program hierarchy of drinking water and sanitation services. Improved drinking water sources include piped water, boreholes or tubewells, protected dug wells, protected springs, rainwater, and packaged water. Improved sanitation facilities include flush/pour flush toilets connected to a sewer system or septic tank, pit latrines with slabs and ventilation, and composting toilets. Adapted from JMP (2021).

Service level	Drinking water	Sanitation
Safely managed	Improved source accessible on premises, available when needed, free from faecal and chemical contamination	Improved facilities that are not shared and excreta are safely disposed in situ or removed and treated off-site
Basic	Improved source, collection time less than 30 minutes for a round trip including queuing	Improved facilities that are not shared with other households
Limited	Improved source, collection time exceeds 30 minutes for a round trip including queuing	Improved facilities that are shared with other households
Unimproved	Unprotected dug well or unprotected spring	Pit latrines without a slab or platform, hanging or bucket latrines
None	Surface water from a river, dam, lake, pond, stream, canal, or irrigation canal	Open defecation, disposal of faeces directly into the environment or with solid waste

2.3.4.1 Water

Schistosomiasis is transmitted to humans through skin contact with water containing cercariae, so if such contact could be completely prevented transmission would stop. Providing access to improved water sources can reduce both the frequency and duration of contact with surface water sources (Farooq et al., 1966; Lima e Costa et al., 1987). However, as the JMP data show, many communities in endemic regions do not have access to improved sources for drinking water, let alone for bathing and laundry (JMP, 2021). Household and community scale water treatment processes such as filtration, chlorination, solar and UV disinfection can be used to treat water collected from contaminated surface water bodies before use, reducing the risk of infection. However, a recent systematic review found that there was insufficient data to develop guidelines for treating water against schistosome cercariae, making it difficult to implement treatment systems that directly target schistosomiasis transmission (Braun et al., 2018).

Community and household piped water has been shown to reduce prevalence, incidence, and intensity of schistosomiasis infections in many locations (Jordan et al., 1975; Jordan et al., 1982; Mason et al., 1986; Sarvel et al., 2011; Ebai et al., 2016). Recent results of a seven year study in rural South Africa found that primary school children living in communities with high piped water coverage were eight times less likely to be infected with urogenital schistosomiasis, and a follow up study found that for every 1% increase in piped water coverage there was a 4.4% reduction in intensity of reinfection following two rounds of treatment with praziquantel (Tanser et al., 2018; Mogeni et al., 2020). Scaling up access to piped water reduced both frequency and duration of contact with contaminated streams among pregnant women in Cameroon, resulting in a decrease in intensity and prevalence of infection (Wepnje et al., 2019). However whilst the risk of infection was essentially eliminated among participants who only used piped water, it had little effect on participants who only partially used piped water (Wepnje et al., 2019). Similarly, the introduction of borehole wells had no effect on incidence of infection in 3 villages in Kenya as whilst use of surface water from drinking, cooking and washing utensils reduced, there was no impact on its use for high contact activities such bathing and laundry (Kholy et al., 1989). These studies highlight that it is not only access to improved water sources but reduction of contact with surface water that is key for preventing schistosomiasis transmission. Many communities

in schistosomiasis endemic regions are also reliant on freshwater bodies for income (e.g. fishing) so completely preventing water contact is unrealistic (Secor, 2014). However, a study in South Africa found that high levels of piped-water coverage provided indirect protection to children who continued to use contaminated water sources, as there was an overall reduction in the parasite reservoir in the community (Tanser *et al.*, 2018). Esrey *et al.* (1991) estimate that providing piped water supply in endemic regions could reduce the global mortality and disease burden of schistosomiasis by 69–77%, although this was based on data from a small number of studies, one of which also included latrine provision.

The effectiveness of water based solutions can be improved by providing communal facilities such as showers, laundry stations, and swimming pools, in addition to standpipes or wells (Taylor *et al.*, 1989; Noda *et al.*, 1997; Kosinski *et al.*, 2012). In a landmark study in St Lucia, household piped water and community shower and laundry facilities were most effective at reducing intensity of infection in children when compared to chemotherapy or snail control, although chemotherapy was more effective at reducing prevalence and incidence of infection (Jordan *et al.*, 1978). However even if extra facilities are provided, they cannot guarantee reduction in contact with surface water and local socio-cultural factors need to be taken into account before implementing water infrastructure schemes. Factors that have been shown to act as barriers to uptake of water infrastructure for contact activities include, but are not limited to:

- Distance to improved water source relative to water bodies (Noda *et al.*, 1997; Abe *et al.*, 2009)
- Overcrowding (Taylor *et al.*, 1987)
- Opportunities for social interaction at local water bodies (Jordan *et al.*, 1978; Acka *et al.*, 2010)
- More privacy at local water bodies for bathing (Cheesmond and Fenwick, 1981; Sow *et al.*, 2004)
- Surface water maybe softer and require less soap and natural stones maybe be preferred to concrete slabs for laundry (Kholy *et al.*, 1989; Chimbari *et al.*, 1992)
- Wash stations and swimming pools maybe muddy and therefore thought to be unclean (Jordan *et al.*, 1978)
- Cultural beliefs that running water is cleaner or "life-giving" (Loroni-Lakwo *et al.*, 1994; Gwatirisa *et al.*, 1999)

Such barriers may be overcome by tailoring water infrastructure projects to suit the local context. Community engagement is required at the early stage of any water infrastructure scheme to ensure it truly meets the need of the community, increasing the likelihood of a long lasting impact.

2.3.4.2 Sanitation

Improving access to sanitation can reduce the number of eggs that are released into freshwater bodies, thereby reducing the number of miracidia and subsequent snail infections. However, because the parasite reproduces asexually within the intermediate host, a reduction in miracidia does not result in a proportional reduction in cercariae (Secor, 2014). Only a few miracidia are required to maintain infections in snails which can produce thousands of cercariae over their lifetime (Gryseels *et al.*, 2006). A systematic review and meta-analysis found that access to sanitation was associated with lower odds of

S. mansoni and *S. haematobium* infection, however only one study included in the review assessed the link between sanitation and *S. japonicum* infection, finding there was no statistical difference between household sanitation and lack of household sanitation (Yang *et al.*, 2015; Freeman *et al.*, 2017). A nationwide survey in Ethiopia found there was no link between school sanitation and a reduction in *S. mansoni* infection (Grimes *et al.*, 2016).

The provision of sanitation does not guarantee universal uptake, and because of the amplifying effect of asexual reproduction within the snail host only a small number of infected people practising open defecation or inadequate latrines is required to maintain transmission throughout a community (Ayad, 1974; Knopp *et al.*, 2013). A study in Zimbabwe found that field workers would not travel long distances to use toilets located in their homes or communities (Chimbari *et al.*, 1992), but people may also just prefer defecating in or around water bodies, possibly because of the availability of water for hygienic bathing (Cheesmond and Fenwick, 1981; Chandiwana, 1986). Preventing urination in the environment is particularly difficult (Shiff, 2017); the majority of *S. haematobium* eggs that reach water are thought to be deposited by children directly urinating into water whilst swimming and bathing (Rollinson *et al.*, 2013). Even if there was universal uptake of sanitation, reservoir animal hosts may deposit eggs into water bodies (although this is only thought to be a significant transmission route for *S. japonicum* (Rollinson *et al.*, 2013; Grimes *et al.*, 2015)) or they may be washed from soiled clothes or the body during bathing (Chandiwana, 1986; Sow *et al.*, 2008).

Whilst it may not be the most impactful intervention for schistosomiasis control, sanitation is a basic human right, and the wider health benefits should not be ignored. Inadequate sanitation disproportionately affects women and girls who may be subjected to sexual harassment when practising open defecation or using unsafe latrines, are more likely to suffer from urinary tract infections, and more likely to avoid school during menstruation if toilets are not available (Devnarain and Matthias, 2011; Koonan, 2019; Kayser *et al.*, 2019). Access to improved sanitation is associated with significantly lower odds of diarrhoeal disease, the second leading cause of mortality in children under the age of five (WHO, 2018a), and it has also been shown to have a positive effect on mental health and well being (Sclar *et al.*, 2018).

2.3.4.3 Hygiene

Hygienic practices that are commonly promoted to prevent diarrhoeal diseases, such as hand washing after defecation, will not have any impact on schistosomiasis transmission because of the mode of infection. However, some types of soap or plant based soap substitutes such as endod can be toxic to cercariae (Okwuosa and Osuala, 1993; Abebe *et al.*, 2005). Increasing the use of soap during laundry and bathing, which are considered high risk activities due to the duration and extent of skin contact, may therefore reduce the risk of infection. A study in Zanzibar found that washing clothes was not significantly associated with prevalence or intensity of *S. haematobium* infection, possibly due to the effects of washing soap (Rudge *et al.*, 2008). Similarly in Ethiopia the prevalence of *S. mansoni* infection dropped by 19% among women and girls following the introduction of endod soap, but not among males who were less likely to be involved in activities such as laundry and therefore less likely to use soap (Erko *et al.*, 2002). Endod soap has the added benefit of being toxic to miricidia and snails and it therefore may have a longer last impact on transmission in addition to providing immediate protection to the user (Abebe *et al.*, 2005).

2.3.5 Health education and behaviour change

Health education plays a key role in amplifying and sustaining all of the inventions listed above and is a key part of the integrated approach to schistosomiasis control (Secor, 2014; WHO, 2020b). Educating communities that are at risk of schistosomiasis improves understanding of the life cycle, symptoms of the disease, and treatment. It has been shown to influence behaviour linked to transmission, such as PC uptake, water contact, environmental management, and use of sanitation facilities and has been associated with a reduction in schistosomiasis prevalence in a number of studies (Aryeetey *et al.*, 1999; Hu *et al.*, 2005; Zhou *et al.*, 2013; Mwanga and Lwambo, 2013; Hürlimann *et al.*, 2018; Martel *et al.*, 2019). It is particularly important to carry out health education campaigns before implementing MDA to combat misinformation or misconceptions regarding the severity of the disease and treatment side effects and ensure high uptake (Muhumuza *et al.*, 2013; Tuhebwe *et al.*, 2015; Inobaya *et al.*, 2018). As with the other interventions, if health education is implemented on its own it can only have a limited effect on the prevalence of schistosomiasis in communities that are reliant on surface water bodies for daily domestic activities (Secor, 2014).

2.3.6 Integrated control

Whilst none of the strategies reviewed here will result in sustainable control and eventual elimination of schistosomiasis if they are implemented on their own, an integrated approach of PC campaigns supported by targeted snail control, improvements in WASH facilities, and comprehensive health education could be more successful, as demonstrated in a number of countries that eliminated schistosomiasis in the 20th century (Rollinson *et al.*, 2013). As the NTD community looks towards elimination of schistosomiasis as a public health problem in 2030, further developments in sensitive point-of-care-diagnostics and disease surveillance and monitoring are also required (Utzinger *et al.*, 2011; Rollinson *et al.*, 2013; Stothard *et al.*, 2017).

The WHO's most recent roadmap for NTDs places a greater focus on multi-sectoral collaboration than ever before and many researchers continue to advocate for such an approach (Utzinger *et al.*, 2003; Rollinson *et al.*, 2013; Waite *et al.*, 2016; Lo *et al.*, 2017; Ross *et al.*, 2017; WHO, 2020b; Boisson *et al.*, 2021). Whilst there are barriers to widespread integration of NTD and WASH programming that need to be overcome, such as funding and differences in indicators and metrics (Freeman *et al.*, 2013; Johnston *et al.*, 2015; Waite *et al.*, 2016), several countries have taken important steps towards embedding collaboration within their national programmes. Dedicated coordination staff and WASH teams have been set up in the NTD programmes in Kenya, Nigeria, and Uganda; Ethiopia has developed a national WASH and NTD framework; and task forces have been set up in Cambodia and Laos to develop community-led initiatives for deworming, nutrition and WASH, demonstrating that effective integration is achievable (Khieu *et al.*, 2019; WHO, 2020b; Boisson *et al.*, 2021).

2.4 Ultraviolet disinfection

Ultraviolet radiation is the region of the electromagnetic spectrum between X-rays and visible light (100–400 nm). It can be categorised into four ranges which relate to the effect these wavelengths have on human skin. Light in the UV-A range (315–400 nm) causes skin tanning whilst the UV-B

range (280–315 nm) causes sunburn and may lead to skin cancer. Wavelengths in the UV-C range (200–280 nm) are absorbed by the nucleic acids and proteins within cells and may cause cell death or mutations (Gray, 2014). The atmosphere strongly absorbs wavelengths less than 290 nm so UV-C light from the sun cannot reach the Earth's surface. Almost all substances, including oxygen, absorb wavelengths below 200 nm, therefore the range from 100–200 nm is referred to as vacuum UV. Between 200 and 300 nm is often referred to as the "germicidal range" as this is most effective for disinfection of microorganisms (Bolton and Cotton, 2008).
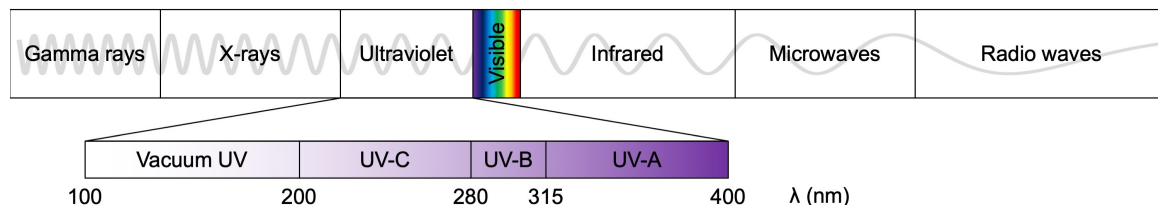
Figure 2.11 Spectral range of ultraviolet light within the electromagnetic spectrum. Image produced in Microsoft® PowerPoint for Mac version 16.56.

2.4.1   History of ultraviolet disinfection of water

There are reports of communities in Asia placing water to be "blessed" by the sun dating back 2000 years (McGuigan *et al.*, 2012) but the anti-microbial properties of sunlight were first scientifically reported by Downes and Blunt in 1877. They found that bacterial and fungal growth in sugar solutions could be inhibited for up to a month in test tubes exposed to sunlight, whereas samples that were kept in the dark would begin growing bacteria and fungi within a few days. Downes and Blunt also reported that disinfection was dependent on the intensity and length of exposure to sunlight, and that fungi appeared to be more resistant than bacteria. They observed in a later study that it was wavelengths at the blue and violet end of the spectrum that were responsible for disinfection (Downes and Blunt, 1878). In the decades that followed, these results were confirmed by a number of researchers who further investigated the effects of different wavelengths on a variety of microorganisms (as reviewed by Hockberger, 2000). By the turn of the century it had been demonstrated that sunlight could be used to disinfect drinking water and that it was actually wavelengths in the UV range that were causing inactivation (Hockberger, 2002).

In 1904 the first quartz mercury arc lamp was made by Richard Küch and between 1904 and 1905 Hertel showed that UV light from arc lamps was much more effective for disinfection than sunlight. Hertel also demonstrated that the order of effectiveness of UV light was UV-C > UV-B > UV-A (Hertel, 1904; Hertel, 1905; as reviewed by Hockberger, 2002; Bolton and Cotton, 2008). This was quickly followed by the first commercial water treatment plant that used UV disinfection to treat filtered river water in Marseilles, France in 1910 (Henri *et al.*, 1910). Due to a number of difficulties the Marseilles plant closed not long after opening and UV disinfection fell out of use following the introduction of chlorination in the 1920's, which was significantly cheaper (Wolfe, 1990). However, as a result of increasing awareness of the harmful disinfection by-products that can be produced by chlorination, the technology was re-embraced in the second half of the 20th century. In 1955 the first modern UV disinfection systems using mercury arc lamps were installed in Switzerland and Austria and by 1990 there were over 2000 UV disinfection plants in Europe, mainly treating groundwater (Wolfe, 1990).

In 1998 Bolton *et al.* made a significant discovery that UV light was highly effective against the chlorine resistant protozoa *Cryptosporidium* spp. This discovery was particularly important in the USA where a series of cryptosporidiosis outbreaks between 1990 and 2000 caused over 100 deaths and illness in hundreds of thousands of people (MacKenzie *et al.*, 1994; Corso *et al.*, 2003), and it resulted in a rapid uptake of UV disinfection in the USA. Unlike previous studies which relied on fluorescent vital and non-vital dyes to determine viability of the oocysts *in vitro*, Bolton *et al.* focused on infectivity, inoculating neonatal mice with the UV exposed oocysts. They demonstrated that high levels of inactivation could be achieved at low UV fluences, when assayed by mouse infectivity, even though *in vitro* assays with the fluorescent dyes showed little or no inactivation after exposure to much higher fluences (Bolton *et al.*, 1998; Bukhari *et al.*, 1999). It has since been found that *in vitro* cell culture assays can also be used to study infectivity of *Cryptosporidium* spp., and that they are generally equivalent to the mouse assay (Rochelle *et al.*, 2002).

Even though the first studies on UV disinfection used sunlight, the potential to harness this free source of UV light for disinfection of drinking water in low-income countries was not investigated until the 1980's. Acra *et al.* (1980) developed a simple technique of using clear, sealable polyethylene bags to expose oral rehydration solutions (prepared with water contaminated with fresh sewage) to sunlight. They found one hour of sunlight was sufficient to reduce the total number of coliforms/mL to zero and that the solutions remained free from coliforms for 24 hours following exposure. The study was followed by more rigorous field and laboratory tests and in 1988 the first workshop on solar disinfection (SODIS) was held in Montreal (Sommer *et al.*, 1997). The Swiss Federal Institute of Aquatic Sciences and Technology (Eawag) began to investigate the method in more detail in the 1990's and in 2001 started implementing SODIS projects in 25 countries. By 2009 it was estimated that more than 4.5 million people relied on SODIS daily for clean drinking water (McGuigan *et al.*, 2012).

A recent innovation that is set to revolutionise UV disinfection are ultraviolet light-emitting diodes (UV LEDs), the discovery of which built on the Nobel Prize winning work of Isamu Akasaki, Hiroshi Amano, and Shuji Nakamura and their development of blue LEDs in the 1980's (Beck, 2018). UV-A LEDs were developed in the 1990's but the first germicidal UV LEDs emitting in the UV-B and UV-C ranges did not become commercially viable until about 2010 (Chatterley and Linden, 2010). Since then there have been rapid advances in efficiency and production costs, meaning UV LEDs are now a viable alternative to mercury arc lamps, with the first full scale UV LED reactor treating 28 mega-litres per day (MLD) installed at a water treatment works in England in 2019 (Jarvis *et al.*, 2019). The different UV sources are reviewed in more detail in Section 2.4.5.

2.4.2 Ultraviolet disinfection mechanisms

UV disinfection is a photobiological process. When microorganisms are exposed to UV light over 95% of photons will pass through the cells but some are absorbed by intracellular molecules (Bolton and Cotton, 2008). Most frequently the absorbed energy from the photon is dissipated but occasionally it causes an electronic interaction. This raises the energy of the molecule from a ground state to an excited state, at which point one of several processes can occur: 1) internal conversion – the molecule returns to its ground state by dissipating the excess energy into the medium as heat; 2) fluorescence/phosphorescence – the molecule returns to its ground state by emitting a photon; or 3) photochemical reaction – the molecule undergoes chemical transformation, producing a photoproduct (Harm, 1980; Bolton and

Cotton, 2008). The biological effectiveness of UV disinfection is primarily attributed to photochemical reactions in the nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Cutler and Zimmerman, 2011). However, UV absorption by proteins and indirect damage caused by the creation of intracellular reactive oxygen species (ROS) may also contribute to inactivation, particularly in the UV-A and UV-B regions (McLaren and Luse, 1961; Jagger, 1981; Eisenstark, 1987).

2.4.2.1 Ultraviolet absorption by nucleic acids

The UV absorbing components of DNA and RNA are the nucleotide bases: the purine derivatives, adenine (A) and guanine (G), and the pyrimidine derivatives thymine (T), cytosine (C), and uracil (U) (which replaces thymine in RNA). Each base has a different absorption spectrum as shown in Figure 2.12. As the base composition of DNA and RNA varies considerably between different microorganism, the absorption spectra of the nucleic acids will also vary, however a common feature is a peak in absorption at approximately 260–265 nm (Harm, 1980). Whilst RNA contains uracil instead of thymine, the absorption spectrum of RNA will generally resemble that of DNA as thymine and uracil have a very similar absorption spectra (Fisher *et al.*, 1974; Harm, 1980; Kowalski *et al.*, 2009).
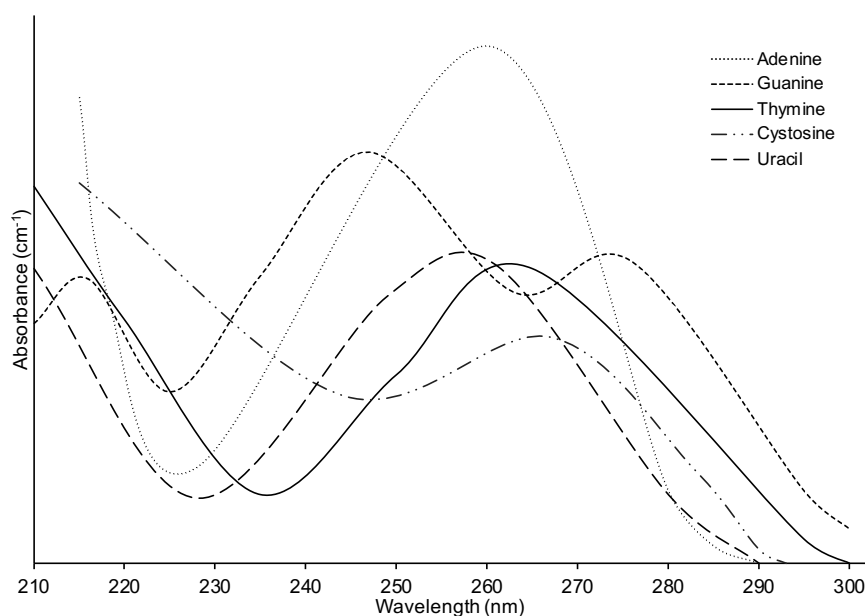
Figure 2.12 Absorption spectra of the nucleotide bases, adapted from Harm (1980) and Bolton and Cotton (2008).

A number of photoproducts may be formed when the nucleic acids absorb a UV photon, but by far the most common and therefore most important in terms of UV disinfection are the cyclobutane pyrimidine dimers (Harm, 1980). A cyclobutane pyrimidine dimer is produced when a new covalent bond forms between two adjacent pyrimidine bases on the same DNA or RNA strand, changing the structure of the nucleic acid. The most common cyclobutane pyrimidine dimers are the thymine-thymine (uracil-uracil in RNA) type but cytosine-thymine and cytosine-cytosine do also occur (Setlow *et al.*, 1963; Setlow and Carrier, 1966; Meistrich, 1972). Other biologically important photoproducts that may be produced in the nucleic acids include pyrimidine adducts, which like cyclobutane pyrimidine dimers are produced by new bonds between adjacent pyrimidine bases, and DNA-protein crosslinks (Wang, 1976; Harm,

1980). However, the formation of thymine-thymine cyclobutane pyrimidine dimers is correlated most strongly with the inactivation of most pathogens (Setlow *et al.*, 1963; Meistrich, 1972). Kowalski *et al.* (2009) have demonstrated that it is possible to predicted the UV sensitivity of some pathogens using genomic modelling. This uses base counting software to estimate the number of potential dimer sites in the pathogens genome based on the overall AT content and the number of adjacent pyrimidine bases. It has so far been used to predict the UV sensitivity of a number of bacteria and viruses, but due to the size of their genomes, it has not been tested on more complex microorganism such as helminths (Kowalski *et al.*, 2009; Kowalski, 2011).

DNA carries the genetic information that is required for a cell to function and replicate. Polymerase enzymes are unable to recognise dimers during transcription and replication of DNA, and if a critical number of dimers are formed it can cause these processes to stall. Preventing DNA replication subsequently prevents cell replication, and in the case of single celled organisms and viruses, this inactivates the pathogen even though it may be metabolically alive (Bolton and Cotton, 2008). Preventing transcription of DNA also results in incomplete messenger RNA molecules which can prevent protein synthesis (Harm, 1980). The consequence of this type of damage is dependent on if the associated gene function is essential or non-essential (Figure 2.13).
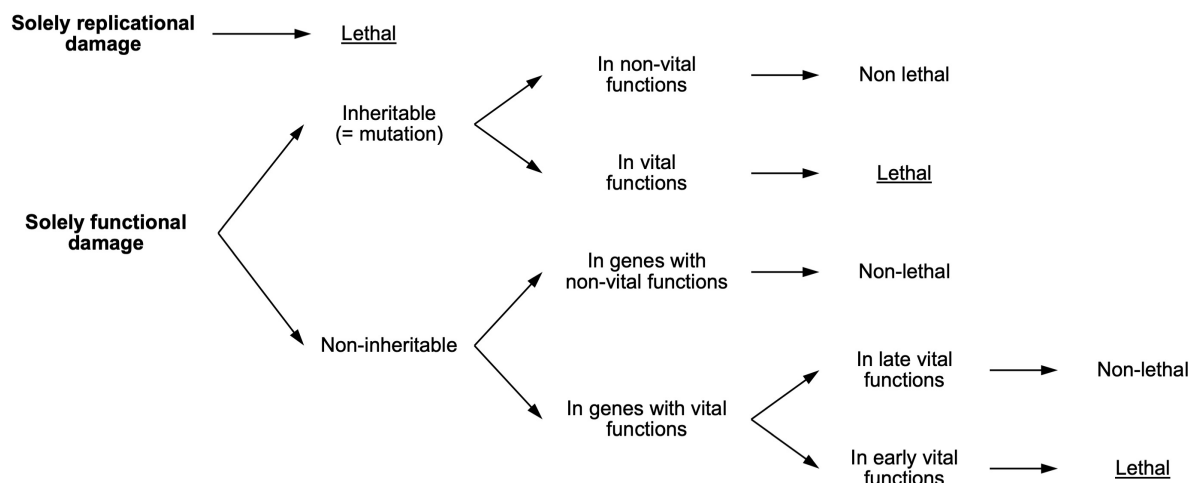
Figure 2.13 Schematic diagram showing the potential outcomes of UV induced nucleic acid damage, adapted from Harm (1980).

Whilst the photobiological properties of RNA are very similar to that of DNA, in most pathogens, UV induced RNA photoproducts are generally considered inconsequential, as unlike DNA, RNA exists in many copies and can be reproduced so long as the DNA is unaffected (Harm, 1980). Exceptions to this include organisms and viruses in which RNA is the only genetic material, such a RNA viruses.

2.4.2.2   Ultraviolet absorption by proteins and other molecules

Absorption of UV light by proteins is generally much lower than that of the nucleic acids (Figure 2.14), as only a few amino acids absorb measurably in the germicidal range (Setlow, 1960). There is strong absorption below 230 nm, which is attributed to the peptide bond, however a smaller peak at

approximately 280 nm is of greater importance for water disinfection as water itself strongly absorbs light below 230 nm and these wavelengths are therefore unavailable for the inactivation of waterborne pathogens (Bolton and Cotton, 2008). UV absorption by proteins in outer cell membranes can disrupt the membranes resulting in leakage of the cytoplasm and cell death. However, as proteins are generally present in very high numbers, high levels of protein damage (and therefore high UV fluences) are generally required for this occur. Damage to proteins has been shown to play an important role in the inactivation of the highly UV-resistant adenovirus (Oguma *et al.*, 2016; Beck *et al.*, 2018) and can also disrupt repair mechanisms (Section 2.4.2.3).
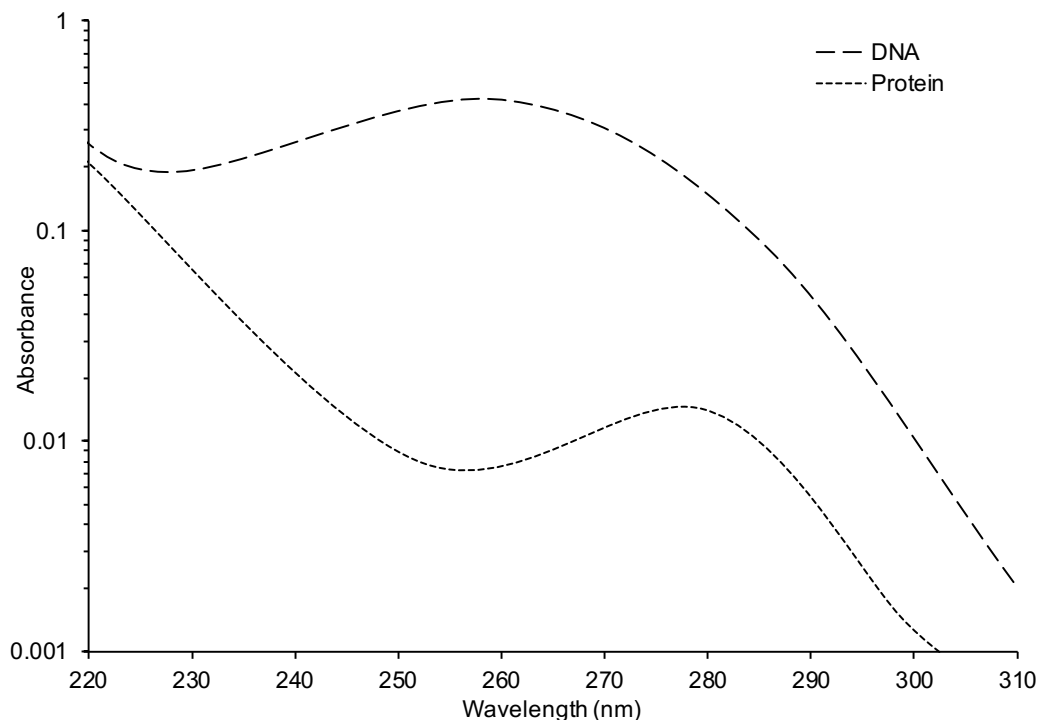
Figure 2.14 Absorption spectra of DNA (calf thymus DNA) and protein (bovine serum albumin) at equal concentration. Adapted from Harm (1980).

Exposure to UV-A and UV-B light stimulates the production of intracellular ROS such as the hydroxyl radical, superoxide, and hydrogen peroxide (McGuigan *et al.*, 2012). These highly reactive molecules can cause damage to the nucleic acids, proteins, and lipids leading to fatal legions in the cell. The origin of intracellular ROS is thought be due to excitation of cytoplasmic proteins and amino acids as well as other endogenous photosensitizers such as porphyrins, flavins, and quinones (Jagger, 1981; Eisenstark, 1987). It has been demonstrated that ROS cause oxidative stress in microorganisms exposed to UV-A and UV-B radiation (Studer *et al.*, 2012; Gerchman *et al.*, 2019; Pousty *et al.*, 2021).

2.4.2.3   Repair mechanisms

At wavelengths less than 280 nm photochemical monomerization of pyrimide dimers can occur if a UV induced dimer subsequently absorbs another UV photon and the photochemical reaction causes it to revert to its original state (Setlow and Setlow, 1962; Pan *et al.*, 2012). However, due to the absorption spectra of the pyrimidine dimers this is most likely to occur in the 230–240 nm regions and requires

very high UV exposure. This mechanism therefore has very little practical impact on water disinfection (Poepping *et al.*, 2014). In contrast, biological repair mechanisms that organisms have developed to protect against harmful exposure to UV light can play a very important role in the UV resistance of some pathogens.

The survival of any species is dependent on the accurate transfer of genetic material from one generation to the next. To achieve this organisms have evolved with the ability to repair DNA damage, whether that be spontaneous damage (e.g. due to inaccuracies during DNA replication) or induced damage (e.g. due to exposure to UV light or carcinogenic substances) (Harm, 1980; Zhou and Elledge, 2000; Sinha and Häder, 2002). There are three known mechanisms capable of repairing UV induced DNA damage: photoreactivation, excision repair, or recombination repair (Harm, 1980). As the name suggests, photoreactivation can only occur in the presence of light. The most frequent type of photoreactivation is photoenzymatic repair, where pyrimidine dimers are monomerized *in situ* by the enzyme photolyase (Rupert, 1962). Triggered by exposure to UV-A and visible light in the blue region, the enzyme binds to pyrimidine dimers in the DNA and reverses the damage. Photolyases have been reported in bacteria, protozoa, and fungi (Kelner, 1950; Oguma *et al.*, 2004; Shin and Linden, 2015), and in more complex organisms including plants, invertebrates, and vertebrates, but the distribution of the enzyme among species and even among the different tissues of a given organism is unpredictable (Sinha and Häder, 2002).

The excision repair mechanism involves a number of enzymes that sequentially cut the DNA, remove and degrade the dimerized nucleotide bases, and then resynthesise the missing section using the intact complementary strand as a template. In the case of recombination repair undamaged DNA regions are replicated and combined such that a complete DNA molecule is formed (Harm, 1980). This is possible because both strands of DNA carry the information for replication. Unlike photoenzymatic repair, excision repair and recombination repair are not specific to UV induced pyrimidine dimers and may be induced by the cell to repair other kinds of DNA alterations. They also do not require light stimulation and therefore are sometimes referred to as dark repair.

UV repair mechanisms require the organisms to be biologically active (although some viruses have been shown to repair UV damage by using the enzymes of their hosts (Eischeid *et al.*, 2009)) and may be inhibited by exposure to high fluences or polychromatic light (Linden and Darby, 1994; Zimmer and Slawson, 2002). Furthermore, repair of the nucleic acids does not necessarily indicate reactivation of the pathogen, particularly if other UV damage mechanisms are present. The protozoan parasite *Cryptosporidium* spp. has been shown to be able to repair UV-induced DNA damage but the oocysts remained non-infective (Oguma *et al.*, 2001). The miracidia of *S. mansoni* have been shown to be able to photoreactivate inside snail hosts (Ruelas *et al.*, 2007), but the repair capabilities of schistosome cercariae have not been investigated in detail. This is discussed in more depth in Sections 3.4 and 5.5.2.

2.4.3 Inactivation kinetics

The theory of UV inactivation kinetics is known as "hit theory" (Hiatt, 1964). A hit is considered to be the formation of a harmful photoproduct that is neither repaired or bypassed. It is best visualised as response curves which represent the reduction in microorganisms on a logarithmic scale as a function

of the UV fluence. The simplest type of theoretical fluence-response is one-hit-one-target, when every increase in fluence decreases the proportion of surviving microorganisms by a constant factor

$$\frac{dN}{dF} = -cN \tag{2.1}$$

where N is the number of surviving microorganisms, F is the fluence, and c is a constant that characterises the UV sensitivity of the microorganisms under the given experimental conditions (Harm, 1980). Integrating over the fluence we obtain

$$\frac{N}{N_0} = e^{-cF} \tag{2.2}$$

in log-linear form this becomes

$$log\left(\frac{N_0}{N}\right) = cF \tag{2.3}$$

where N_0 is initial number of microorganisms. Whilst experimental data may be evaluated in terms of one-hit-one-target kinetics, in reality this is rarely observed in nature as most microorganisms have some repair capability resulting little or no response to very low fluences (Harm, 1980). This is often described as the shoulder region of a fluence-response curve. There are two types of theoretical shouldered response curves: multi-target where the organism contains n distinct targets, each of which must receive a hit to become inactivated, and multi-hit where a single target must be hit at least n times (Figure 2.15a) (Hiatt, 1964; Severin *et al.*, 1983). Shouldered curves are frequently observed in experimental data, most likely due to repair mechanisms which become less effective at higher fluences, rather than the theoretical mechanisms described here. However, true multi-target curves may be observed in cells or organisms that contain multiple sets of genetic material or in samples where suspended cells exist in small groups and all individuals must be inactivated to achieve inactivation or a loss of colony formation (Harm, 1980).

Multi-component curves, which form a tailing shape, may be observed in samples that contain two or more sub-populations which have different sensitivities to UV light (Figure 2.15b). Harm (1980) provides the example of two bacteriophages T1 and T3, one of which undergoes extensive repair and therefore appears less sensitive. Tailing shaped curves may also be caused by shielding of microorganism by particles or other microorganism higher in the water column (Templeton *et al.*, 2008).
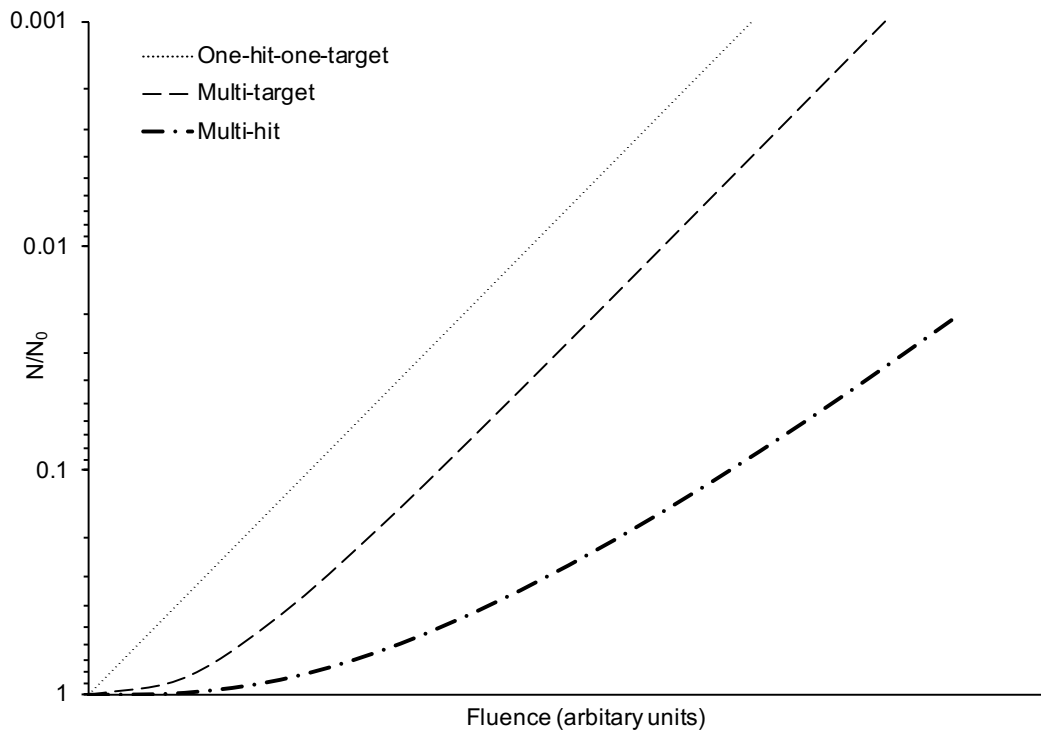
(a)
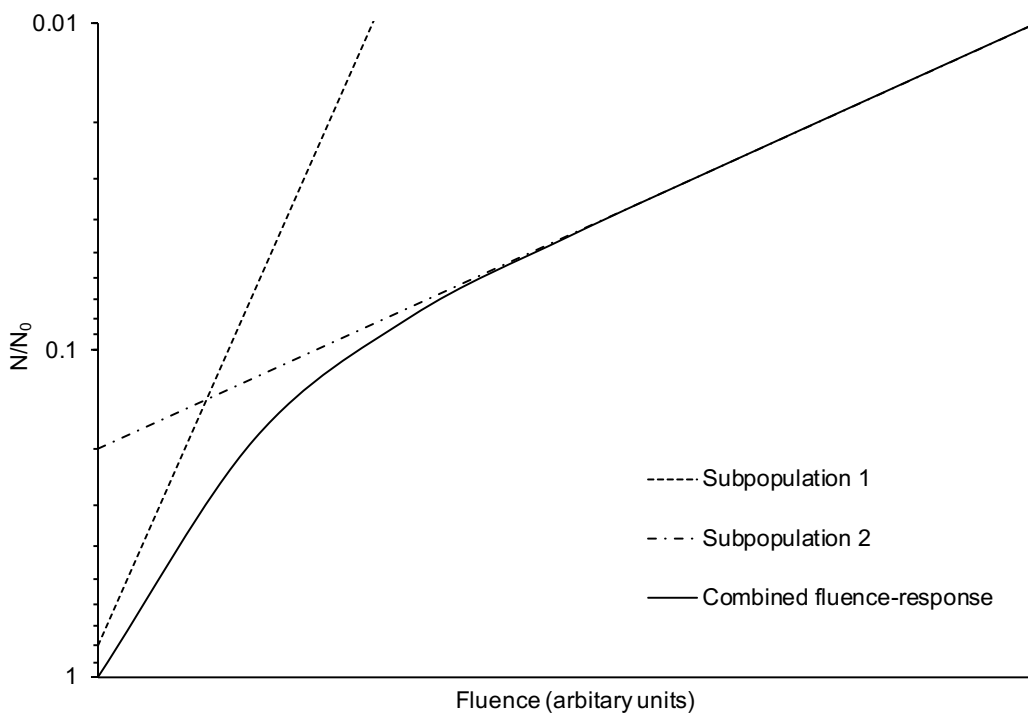
(b)

Figure 2.15 (a) A comparison of one-hit-one-target, multi-target, and multi-hit fluence-response curves and **(b)** an example of a multi-component fluence-response curve of two sub-populations of microorganisms, each with a different ultraviolet sensitivity. Both adapted from Harm (1980).

2.4.4 Ultraviolet sensitivity of pathogens

Microorganisms exhibit a wide range in sensitivity to UV light which is dependent on a number of factors including, but not limited to: nucleic acid composition, physical size, repair capabilities, surface structure, and the wavelength of light the organism was exposed to (Masjoudi *et al.*, 2021). The general order of UV sensitivity of pathogens from most to least sensitive is bacteria $\approx$ protozoa > viruses > bacterial spores > algae (Bolton and Cotton, 2008), however there are some exceptions, such as adenovirus, a highly UV resistant respiratory virus. The fluence required to achieve a 4-$\log_{10}$ reduction of some common waterborne pathogens are listed in Table 2.4. There has been relatively little research into the UV sensitivity of helminths, which is why they are not included in this list. The UV sensitivity of WASH-related helminths is discussed in detail in Chapter 3.

Table 2.4 Fluence required for a 4-$\log_{10}$ reduction of some waterborne pathogens at 253.7 nm, without photoreactivation (i.e. sample were kept in the dark following exposure). Adapted from Masjoudi *et al.* (2021).

	Fluence (mJ/cm^2) for 4-$\log_{10}$ inactivation	References
Bacterium		
Vibrio cholerae (ATTC 25872)	2.9	Bolton and Cotton (2008)
Escherichia coli wild type	8.1	Sommer *et al.* (2000)
Bacillus subtilis spores (ATCC 6633)	62 ± 11	ÖNORM (2001)
Protozan (oo)cysts		
Giardia lamblia	< 2	Linden *et al.* (2002) and Mofidi *et al.* (2002)
Cryptosporidium parvum	9.5	Craik *et al.* (2001)
Virus		
Hepatitis A (HM175)	16	Battigelli *et al.* (1993)
Adenovirus Type 40	130	Thurston-Enriquez *et al.* (2003) and Blatchley *et al.* (2008)

2.4.5 Sources of ultraviolet light

2.4.5.1 Mercury arc lamps

The most widely used sources of UV light for water disinfection are mercury arc lamps. These lamps are comprised of two electrodes at each end of a quartz tube that contains mercury gas. They emit light when the mercury atoms are excited to higher energy states by electrons emitted from the electrodes, as the atoms return to their ground state they emit a UV photon (Bolton and Cotton, 2008). Mercury arc lamps produce UV light with a fixed spectral emission pattern which is dependent on the pressure of the mercury gas. Low pressure mercury arc lamps (LP lamps) produce near-monochromatic light at 253.7 nm, which is close to the absorption maximum of DNA, hence why these lamps are sometimes referred to as germicidal lamps. Medium pressure mercury arc lamps (MP lamps) emit polychromatic light over a broad spectrum (Figure 2.16). The main advantage of MP lamps is that the optical output is 20–50 times higher than that of LP lamps of the same length. However this advantage is diminished by a lower spectral efficiency, with only 5–15% of emitted light is in the UV-C range, and shorter lamp life (Schalk *et al.*, 2006). MP lamps are therefore primarily used in water treatment plants with very high flow rates as fewer lamps are required, reducing the operating costs. Other advantages of MP lamps include inhibition of photoreactivation in some microorganisms and better effectiveness against

adenovirus. This has been attributed to protein damage in enzymes and other biologically important structures, such as the viral capsid, following exposure to polychromatic light (Zimmer and Slawson, 2002; Eischeid and Linden, 2011; Beck *et al.*, 2018).
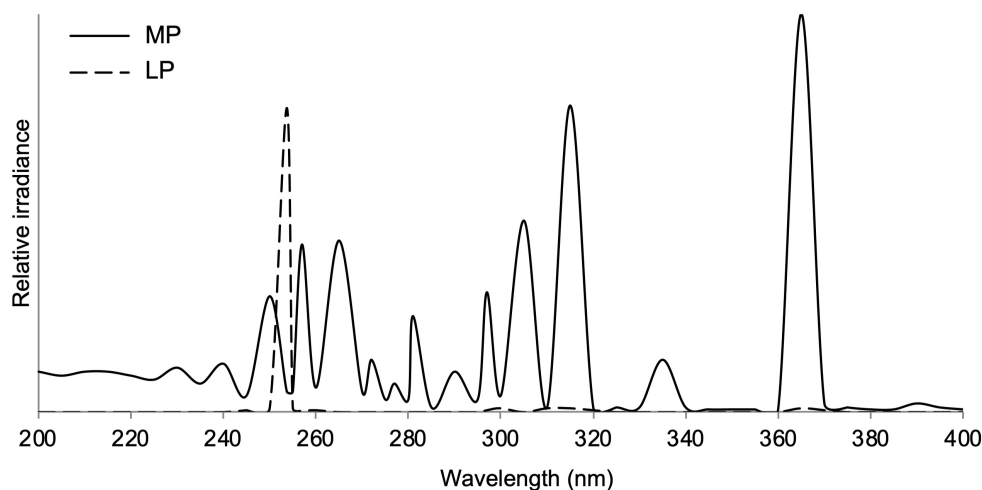
Figure 2.16 Typical spectral power distributions of low pressure (LP) and medium pressure (MP) mercury arc lamps, adapted from Bolton and Cotton (2008).

2.4.5.2 Ultraviolet light-emitting diodes

Light-emitting diodes consist of two types of semiconductor, a p-type and a n-type, that are bound one above the other to a stable substrate. The two types are formed by doping a semiconducting material with impurities that have either one less or one more valence electrons (p- and n-type, respectively). When a voltage is applied the free electrons flowing from the n-region "fall" into the electron holes in the p-region and in doing so enter a lower energy state. The excess energy is released in the form of polychromatic light in a relatively narrow spectral range (full width half maximum (FWHM) typically 10–15 nm, see Figure 4.3), the distribution of which depends on the energy difference between each state. This energy difference is known as the bandgap and it is a property of the semiconducting material (Chen *et al.*, 2017; Beck, 2018). UV LEDs can therefore be manufactured to emit light at various peak wavelengths by using different types of semiconducting material to manipulate the bandgap. This means disinfection systems can be "tuned" to specific peak wavelengths that coincide with the action spectra of target organisms. Recent studies comparing UV LEDs with conventional UV disinfection technology have found that UV LEDs with peak wavelengths between 260 nm and 290 nm are more effective at inactivating some bacteria, fungi, and viruses than LP lamps. This has been attributed to a range of effects including suppression of photoreactivation mechanisms, increased protein damage in cell membranes, and increased production of intracellular ROS (Sholtes *et al.*, 2016; Beck *et al.*, 2017; Li *et al.*, 2017; Rattanakul and Oguma, 2018; Woo *et al.*, 2019; Wan *et al.*, 2020).

Additional advantages of UV LEDs are that they are mercury-free, durable, and small. They are well suited to innovative, point-of-use applications such as fitting them into taps, pumps, or water bottles (Song *et al.*, 2016; Linden *et al.*, 2019; Sundar and Kanmani, 2020). They also have near instantaneous start up time (mercury arc lamps need to warm up for at least 15 minutes to ensure a stable output), an ambient surface temperature which does not promote fouling of the reactor, and much longer lifetimes

than LP and MP lamps (Chen *et al.*, 2017).

The optical power of UV LEDs is currently relatively low compared to mercury arc lamps, meaning longer exposure times may be required to achieve sufficient inactivation of some pathogens. The wall plug efficiency (WPE, ratio of optical output power to electrical input power) of LP lamps is 30–40% (Ibrahim *et al.*, 2014), compared to approximately 10% for the best available UV LEDs in the germicidal range (Zhang *et al.*, 2019; Krames, 2020). However, this is an increase from 1% in 2015, with predictions suggesting that the WPE of UV LEDs at 260—280 nm could exceed 20% by 2025, assuming they follow the trend of blue LEDs (Kneissl *et al.*, 2019; Krames, 2020). Beck *et al.* (2017) suggest that UV LEDs must reach a WPE of 25–39% to match the electrical efficiency of LP lamps. Nevertheless, the cost of germicidal UV LEDs has decreased from over 1000 USD/mW in 2003 to less than 1 USD/mW today (Lawal *et al.*, 2018), with predictions they will be commercially available for less than 1 USD/W by 2030. For comparison at 10 USD/W blue LEDs had already entered the television market (Krames, 2020).

2.4.5.3 Solar disinfection

SODIS is widely recognised as a sustainable form of household level water treatment that has been shown to be effective against a wide range of waterborne bacteria with varying effectiveness against viruses and protozoa (Conroy *et al.*, 2001; Bosshard *et al.*, 2009; Heaselgrave and Kilvington, 2011; Amin *et al.*, 2014; Luzi *et al.*, 2016). The basic SODIS technique typically involves filling 2-litre polyethylene-terephthalate (PET) drinks bottles with water and placing them in direct sunlight for a minimum of six hours, increasing the exposure time to 24 hours on cloudy days (Luzi *et al.*, 2016). The main benefit of SODIS is that it has no operational costs once the bottle or reactor has been obtained, it is therefore particularly applicable for low income regions. An added benefit of using PET bottles as the SODIS reactor is that water can be drunk directly from the bottle, which reduces the chance of recontamination. This is of particular importance for UV disinfection as it does not provide residual protection, unlike chlorination. Studies have investigated scaling up the technology with flow-through reactors, typically made of borosilicate glass tubes (Chaúque and Rott, 2021), however the downside of such systems is that the key advantages of SODIS, cost effectiveness and simplicity, are lost.

The disinfection mechanism is a combination of UV-A and UV-B disinfection and solar heating (McGuigan *et al.*, 1998). Shaking a two-thirds filled bottle for 30 seconds before filling up and sealing can improves efficiency by increasing initial levels of dissolved oxygen which can react with UV-A photosensitizers present in surface waters to produce extracellular ROS (Kehoe *et al.*, 2001; McGuigan *et al.*, 2012; Luzi *et al.*, 2016). Disinfection can also be enhanced by filtering water before filling the bottles, placing the bottles on reflective surfaces, or painting the underside of the bottles black to enhance solar heating (Sommer *et al.*, 1997; Kehoe *et al.*, 2001; Martín-Domínguez *et al.*, 2005).

2.4.6 Fluence determination

In the water treatment industry UV exposure is quantified in terms of fluence (mJ/cm^2). Fluence is defined as the incident radiant energy per unit area passing through an infinitely small sphere of cross-sectional area dA, from all directions. The term "UV dose" is often used interchangeably with fluence, however fluence is the correct term when discussing the inactivation of microorganisms. This is because

"dose" may imply all the applied energy is absorbed, but in reality almost all of the incident UV energy passes through microorganisms, with only a small amount being absorbed. In order to understand how fluence is calculated, two other key terms must be defined: fluence rate and irradiance.

Fluence rate (mW/cm^2) is the incident radiant power per unit area passing through an infinitely small *sphere* of cross-sectional area dA, from *all directions*. If a microorganism is exposed to a constant fluence rate, the fluence is a product of the fluence rate and exposure time (s). Irradiance is defined as the incident radiant power per unit area from *all upward directions* on an infinitely small *surface* of the area dS. For a parallel and perpendicular (collimated) beam the irradiance is equal to the fluence rate and under the correct experimental conditions, the fluence rate applied to a water sample can be approximated using a radiometer to measure the irradiance. It is important to note that fluence rate is the correct term for discussing the inactivation of microorganisms as they can receive UV light from any direction.

The bench scale "collimated beam" was designed to enable fluence rate estimation for mercury arc lamps and a standardised method for fluence determination using this apparatus was published by Bolton and Linden in 2003. The purpose of the collimating tube was to provide an uniform irradiance field over the surface area of a sample suspension, typically in a Petri dish. A minimum distance of four times the aperture diameter was required to achieve a uniform distribution, at which point the incident radiation was found to be approximately collimated and therefore the fluence rate could be estimated by measuring the irradiance. As a radiometer can only measure the incident irradiance at the centre of the sample surface, the following correction factors were applied by Bolton and Linden (2003) to obtain the average irradiance in the water, and hence an estimate of the average fluence rate to which each microorganism is exposed:

- Petri factor – evaluates the uniformity of the incident radiation over the sample surface area, it is defined as the ratio of the average irradiance across the sample surface to the irradiance at the centre of the sample.

- Water factor – accounts for the decrease in irradiance due to absorption by the water column.

- Divergence factor – as the beam is not perfectly collimated, this accounts for divergence over the sample depth and is defined ratio of the average irradiance inside the Petri dish in the absence of the sample to the average irradiance at the sample surface.

- Reflection factor – accounts for the reflection of a fraction of the beam from the water surface due to the different refractive indices of air and water.

- Sensor factor (polychromatic sources only) – accounts for the variation of the sensitivity of the radiometer detector over the spectral power distribution of the UV source.

- Germicidal factor (polychromatic sources only) – as the aim of the Bolton and Linden protocol was to measure the germicidal UV irradiance this factor weights each wavelength to its relative germicidal effectiveness as determined by the action spectra of the target microorganism, if this is unknown the action spectra of DNA is often used as a substitute .

The Bolton and Linden protocol was adopted by the International Ultraviolet Association and the United States Environmental Protection Agency (USEPA) as a standard protocol for fluence determi-

nation for mercury arc lamps. However it is not directly applicable to UV LEDs which are low power and have non-uniform distribution patterns (that are typically balloon shaped or heart shaped) (Chen *et al.*, 2017). At the distances required to achieve collimated light from a mercury arc lamp the incident irradiance of UV LEDs would be too low to achieve meaningful inactivation within a reasonable time frame. Early studies using UV LEDs therefore used much shorter distances between the source and sample surface, however at such distances the light may not be collimated due to wide viewing angles (normally over 100°) (Kheyrandish *et al.*, 2018).

To account for these differences Kheyrandish *et al.* (2018) devised a new protocol for UV LED fluence determination. The main difference between the UV LED protocol and LP protocol is how the uniformity of the irradiance distribution is calculated. Some UV LEDs have heart shaped irradiance patterns (i.e. the peak irradiance is not in the centre of the Petri dish) which can result in meaningless Petri factors that are greater than or equal to one. Therefore Kheyrandish *et al.* (2018) suggested measuring the irradiance distribution on a polar grid and calculating the weighted average irradiance on the sample surface. The uniformity could then be evaluated by calculating the coefficient of variation (CV). A CV of 6.7% or more would be equal to a Petri factor greater than 0.9. This method is discussed in more detail in Section 4.3.4.

2.4.7 Ultraviolet disinfection and water, sanitation, and hygiene interventions

Possibly the most widely researched form of UV disinfection in WASH programmes is SODIS, which is targeted specifically at low income communities with limited resources (McGuigan *et al.*, 2012; Marugán *et al.*, 2020). It has has been used to treat water from a variety of sources including rainwater, surface water, and contaminated tap water (Mustafa *et al.*, 2012; Amin *et al.*, 2014; Islam *et al.*, 2015). A number of field studies have demonstrated the positive effect of SODIS on child health in particular (Conroy *et al.*, 1996; Conroy *et al.*, 2001; Rose *et al.*, 2006; Rai *et al.*, 2010; Marugán *et al.*, 2020), and a meta-analysis of 10 eligible studies estimated that SODIS has the potential to reduce incidence of diarrhoea by 38% (Soboksa *et al.*, 2020). There are limited data from randomised controlled trials, however two such studies in Kenya and Cambodia reported statistically significant reductions of dysentery and diarrhoea among children who drank SODIS water compared to controls (Preez *et al.*, 2011; McGuigan *et al.*, 2011). The study in Kenya also reported significantly higher median height-for-age in SODIS intervention arms than in control groups, and the median weight-for-age was also higher, although the difference was not significant (Preez *et al.*, 2011).

Studies have reported considerable variations in uptake and compliance of SODIS between 4% and 90% (Arnold *et al.*, 2009; McGuigan *et al.*, 2011; Islam *et al.*, 2015; Nuño Martínez *et al.*, 2020), however a common pattern is high initial compliance that significantly reduces overtime. A randomised controlled trial in South Africa reported a reduction from 75% compliance to <30% after 40 weeks, and hardly any participants continued to use SODIS a year after the study had ended (Preez *et al.*, 2010). Barriers to uptake are not necessarily exclusive to SODIS and include lack of understanding about the necessity of treating water and habit or preference of using other water sources (Rainey and Harding, 2005; Nuño Martínez *et al.*, 2020). However studies have found that some users do not like the taste, smell, or temperature of SODIS treated water (Tamas and Mosler, 2011; Nuño Martínez *et al.*, 2020). Users may also lack confidence in the SODIS method or find it too labour intensive and time consuming, particularly if one person is responsible for treating water for an entire household (Rainey and Harding,

2005; Christen *et al.*, 2011; McGuigan *et al.*, 2012; Borde *et al.*, 2016; Nuño Martínez *et al.*, 2020). Another significant barrier to increased uptake of SODIS is the concern that sunlight exposure leads to the leaching of toxic substances from the plastic bottle into the water (McGuigan *et al.*, 2012). Whilst some studies have shown that SODIS can slightly increase the migration of some organic substances from PET bottles into water, the concentrations were well below the WHO limits for drinking water and in the same order of magnitude as controls that were kept in the dark (Schmid *et al.*, 2008; Mustafa *et al.*, 2012; Bach *et al.*, 2013; Bach *et al.*, 2014). Nevertheless this psychological barrier needs to be addressed if SODIS is to be more widely adopted (McGuigan *et al.*, 2012). Repeated promotion and training programmes have been found to be particularly important in sustaining use of SODIS (Rainey and Harding, 2005; McGuigan *et al.*, 2011; Christen *et al.*, 2011).

SODIS is typically carried out in 2 L PET bottles, which are well suited for drinking water, but it is estimated that an absolute minimum of 7.5–15 L of water per day are required to fulfil all basic water needs, depending on climate, sanitation facilities, and cultural norms (WHO, 2011). PET bags which can hold up to 7 L, large 19 L polycarbonate water dispenser bottles, and 20 L transparent polypropylene buckets have been shown to be effective SODIS reactors, and maybe more suitable for water contact activities such as hand washing and laundry (Lawand *et al.*, 1997; Keogh *et al.*, 2015; Borde *et al.*, 2016; Polo-López *et al.*, 2019). However there are no studies investigating the use of these large SODIS reactors for water contact activities nor the impact of SODIS on disease transmission routes other than via drinking water. Given that SODIS relies on a combination of UV-A and UV-B disinfection and solar heating, lack of investigation of SODIS for activities that benefit from using warm water seems like a missed opportunity.

Community and household scale UV disinfection units that utilise mercury arc lamps have been implemented in low- and middle-income countries throughout Latin America, Africa, and Asia (Gadgil *et al.*, 1998; deWilde *et al.*, 2008; Opryszko *et al.*, 2013; Hunter *et al.*, 2013; Barstow *et al.*, 2014; Maciel *et al.*, 2021). Brownell *et al.* (2007) demonstrated that a simple, household scale flow-through reactor could be built for less than 50 USD using locally available materials, and household scale reactors based on this design were distributed in rural Mexico for a cost to the consumer of 20–24 USD per unit (Gruber *et al.*, 2013; Reygadas *et al.*, 2018). The simple reactor was gravity fed and used a narrow necked bottle to store treated water to reduce the risk of recontamination post-treatment (Reygadas *et al.*, 2018). Whilst these systems increased the number of households that had access to safe water, there was no significant reduction in diarrhoea, possibly because of unexpectedly low diarrhoea prevalence in control households, recontamination from drinking glasses, or because participants continued to consume untreated water alongside treated water (Reygadas *et al.*, 2015). User compliance also reduced over time, even in communities that were provided with additional support and health education, however the reasons for non-compliance such as habit or convenience of using other untreated water sources were not specific to UV disinfection (Reygadas *et al.*, 2018).

Small water enterprises that sell UV treated water for-profit have been shown to be a sustainable way of providing community scale UV water treatment (Gadgil and Derby, 2003; Opryszko *et al.*, 2013; Hunter *et al.*, 2013). Local entrepreneurs are supported by NGOs or local government to build water treatment plants that use filtration and UV disinfection to treat contaminated groundwater or surface water. Quality control is carried out by technicians from the NGO who also provide technical assistance to the vendors when required. The treatment plants can be solar powered and the cost to consumers

can be as little as 0.01 USD per litre (Opryszko *et al.*, 2013; Hunter *et al.*, 2013). Such programme have been shown to reduce the risk of diarrhoea in children and, when the water was provided in schools free of charge, there was a reduction in absenteeism (Hunter *et al.*, 2013; Hunter *et al.*, 2014). As UV treatment does not provide a disinfection residual recontamination from dirty containers and improper storage can reduce the effectiveness of these interventions (Opryszko *et al.*, 2013), but this can be avoided by providing the water to consumers in clean containers which are then returned to the vendor for disinfection and subsequent refilling (Hunter *et al.*, 2013). Alternatively UV disinfection can be combined with chlorination to ensure a disinfection residual whilst protecting against chlorine resistant pathogens such as *Cryptosporidium* spp., although this may impart a taste or odour to the water, which has been shown to reduce the acceptability of chlorinated water in low- and middle-income countries (Jeuland *et al.*, 2016; Crider *et al.*, 2018; Smith *et al.*, 2021; Maciel *et al.*, 2021).

As a relatively new technology, the capital costs of UV LED treatment systems are currently too high for them to be widely adopted in WASH programmes, however a number of pilot studies have demonstrated that they are well suited to point-of-use or community scale water treatment, requiring little to no maintenance with very low operational costs (Chatterley and Linden, 2010; Hull *et al.*, 2019; Hadjeres *et al.*, 2019). They have also been shown to be an effective, low-cost form of wastewater treatment suitable for low-income countries (Nguyen *et al.*, 2019). A recent study in India evaluated a "zero-energy" UV LED reactor to treat water from an unprotected well. A dynamo was used to convert the mechanical energy generated by hand pumping water to electrical energy for operating the UV LEDs (Sundar and Kanmani, 2020). The capacity of the reactor was only 1 L however the study demonstrates the potential of UV LEDs to revolutionise water disinfection in low-income regions, something that has been noted by a number of researchers in the UV field (Ibrahim *et al.*, 2014; Lui *et al.*, 2014; Loeb *et al.*, 2016; Linden *et al.*, 2018; Linden *et al.*, 2019).

3 Systematic review of the ultraviolet sensitivity of WASH-related helminths

3.1 Abstract

Helminthiases are a group of disabling NTDs that affect billions of people worldwide. Current control methods use preventative chemotherapy but reinfection is common and an integrated approach is required if elimination is to be achieved. Household and community scale water treatment can be used to provide a safe alternative water supply for contact activities, reducing exposure to WASH-related helminths. UV LEDs could be a realistic option for water treatment in low-income regions in the near future, but currently there is no guidance for the use of UV or solar disinfection against helminths. A qualitative systematic review of existing literature was carried out to establish which WASH-related helminths are more susceptible to UV disinfection and identify gaps in the research to inform future studies. The search included all species that can infect humans and can be transmitted through water or wastewater. Five online databases were searched and results were categorised based on the UV source: sunlight and solar simulators, UV-A and UV-B (long wavelength) sources, and UV-C (germicidal) sources. There has been very little research into the UV sensitivity of helminths; only 47 studies were included in this review and the majority were carried out before the standard protocol for UV disinfection experiments was published. Only 18 species were studied; however all species could be inactivated by UV light. Fluences required to achieve a 1-$\log_{10}$ inactivation ranged from $5\,\mathrm{mJ/cm^2}$ to over $800\,\mathrm{mJ/cm^2}$. Larval forms, including schistosome cercariae, were generally more sensitive to UV light than species which remain as an egg in the environment. This review confirms that further research is required to produce detailed recommendations for household or community scale UV LED or SODIS of water for preventing helminthiases.

3.2 Introduction

In 2016, WASH-related helminth infections, including schistosomiasis, were responsible for over 7.7 million DALYs (GBD 2016 Collaborators, 2017). These diseases are transmitted through contact with (or consumption of) water, food, and soil that contain the human infective stages of the parasite (CDC, 2019). Current control methods are primarily focused on PC with anthelmintic drugs, but reinfection is common. Household and community scale water treatment processes can be used to treat water collected or abstracted from contaminated water bodies. This reduces exposure to helminth eggs and larvae by providing safe alternative water supplies for contact activities such as hand washing, bathing, and laundry and if treated sufficiently the water may also be used for drinking.

UV disinfection with mercury arc lamps and SODIS are already used to treat water in many countries that are endemic to WASH-related helminths (Section 2.4.7), and the recent rapid development of UV LEDs could lead to further innovation for water treatment in low-income regions. However, whilst these water treatment processes have been shown to be effective against a wide range of bacteria, viruses,

and protozoa (Masjoudi *et al.*, 2021), there is no formal guidance for using UV disinfection against helminths, even though many can be spread through water. Although "helminth" is a generic term used to describe a group of varied organisms that may not be related, parasitic worms are physically similar, particularly when compared to single celled pathogens, and often have common characteristics (e.g. complex life cycles involving one or more host). Examining the UV disinfection literature on this broad group of organisms may therefore help to improve the understanding of the UV sensitivity and disinfection mechanism of a specific family or genus of helminths, such as schistosomes. Hence, the aim of this chapter is to summarise the existing knowledge on WASH-related helminths and UV disinfection; investigate any similarities in possible disinfection mechanisms; and determine which species are more susceptible to this form of water treatment. The review also identifies gaps in the research which will inform future studies regarding the proper use of UV and SODIS for minimising the spread of these diseases via water in low-income regions.

3.3   Methods

This systematic review follows the guidelines of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (Moher *et al.*, 2009). The search took place between 1 and 6 June 2018 and included five databases: Web of Science, PubMed, The British Library, Scopus, and Google Scholar. All languages and document types were included, and databases were searched from inception to present day. The databases were searched for any combination of species name (Table 3.1) and common UV disinfection terms (UV, Ultra-violet, Ultraviolet, SODIS, Sunlight, Solar Disinfection) in the title. The search included all WASH-related helminths that can infect humans (including zoonotic species) as listed on the Centre for Disease Control index of Parasites of Public Health Concern (CDC, 2019). This includes species that are not necessarily waterborne but that can be transmitted through water if they enter water or wastewater (such as STHs which are spread through faeces). Class names cestode, nematode, and trematode, and common names, such as hookworm, were also included in the search. The term herpes was excluded from the search as is returned a large number of irrelevant studies.

For example, the search of the Web of Science database was as follows:

TI = (Ancylostom* OR brazilense OR caninum OR ceylanicum OR duodenale OR Necator OR americanus OR Uncinaria OR stenocephala OR Hookworm OR Angiostrongyl* OR cantonensis OR costaricensis OR Parastrongyl* OR Anisaki* OR simplex OR Pseudoterranova OR decipiens OR Ascari* OR lumbricoides OR suum OR Roundworm OR Capillaria* OR hepatica OR philippinensis OR aerophila OR Clonorchi* OR sinensis OR Diphyllobothri* OR latum OR pacificum OR cordatum OR ursi OR dendriticum OR lanceolatum OR dalliae OR yonagoensis OR Spirometra OR mansonoides OR erinacei OR ranarum OR Dracuncul* OR medinensis OR Guinea worm OR Echinococc* OR granulosus OR multilocularis OR vogeli OR oligarthrus OR Hydatid OR Fasciol* OR hepatica OR gigantica OR Fasciolopsi* OR buski OR Gnathostom* OR spinigerum OR hispidum OR Heterophy* OR heterophyes OR Metagonim* OR yokogawai OR Hymenolepi* OR nana OR Opisthorchi* OR viverrini OR felineus OR Paragonim* OR westermani OR Schistosom* OR mansoni OR haematobium OR japonicum OR Bilharz* OR Taenia OR solium OR saginata OR asiatica OR Tapeworm OR cysticercosis OR Trichuris OR trichiura OR Whipworm OR Helminth OR STH OR Trematode OR Cestode OR Nematode OR Acanthocephalan OR Flatworm OR Fluke) AND TI = (UV OR Ultra-violet OR Ultraviolet OR SODIS OR Sunlight OR Solar Disinfection) NOT TS=(herpes)

Table 3.1 List of species included in the database search, grouped by class.

Cestodes	Nematodes	Trematodes
Diphyllobothrium latum	*Ancylostoma brazilense*	*Clonorchis sinensis*
Diphyllobothrium pacificum	*Ancylostoma caninum*	*Fasciola hepatica*
Diphyllobothrium cordatum	*Ancylostoma ceylanicum*	*Fasciola gigantica*
Diphyllobothrium ursi	*Ancylostoma duodenale*	*Fasciolopsis buski*
Diphyllobothrium dendriticum	*Necator americanus*	*Heterophyes heterophyes*
Diphyllobothrium lanceolatum	*Uncinaria stenocephala*	*Metagonimus yokogawai*
Diphyllobothrium dalliae	*Angiostrongylus cantonensis*	*Opisthorchis viverrini*
Diphyllobothrium yonagoensis	*Angiostrongylus costaricensi*	*Opisthorchis felineus*
Spirometra mansonoides	*Parastrongylus costaricensis*	*Paragonimus westermani*
Spirometra erinacei	*Ascaris lumbricoide*	*Schistosoma mansoni*
Spirometra mansoni	*Ascaris suum*	*Schistosoma haematobium*
Spirometra ranarum	*Anisakis simplex*	*Schistosoma japonicum*
Echinococcus granulosus	*Pseudoterranova decipiens*	
Echinococcus multilocularis	*Capillaria hepatica*	
Echinococcus vogeli	*Capillaria philippinensis*	
Echinococcus oligarthrus	*Capillaria aerophila*	
Hymenolepis nana	*Dracunculus medinensis*	
Taenia solium	*Gnathostoma spinigerum*	
Taenia saginata	*Gnathostoma hispidum*	
Taenia asiatica	*Trichuris trichiura*	

3.3.1 Classification criteria

The studies were reviewed and classified according to the flow chart in Table 3.2. First duplicates were removed and assigned code 1, then the papers were classified by title. Titles which suggested the studies were not about a relevant species or UV disinfection were removed and assigned codes 2-4 (animal species of the listed genera were included). The remaining abstracts were read and those that were about a non-waterborne life stage or primarily about the host response to a UV-attenuated vaccine were assigned codes 5 and 6, and removed. Papers for the remaining studies were obtained and read in full. Studies that provided limited information about the effect of UV light on the helminth or that contained significant errors were assigned codes 7 and 8 and were excluded; the remainder (code 9) were included in the review. Additional studies that were referenced in the papers in a way that suggested they were relevant to this review were also obtained, read in full, and assigned the relevant code. Papers were obtained from Imperial College London Library, The British Library, and The Wellcome Trust. Papers that were not written in English were either translated using online translation software (Google Docs translation tool) or by Imperial College London students who were native speakers of the language. Notes were made on the studies and relevant information was extracted and included in a table (Appendix A). Studies were also read independently by former Imperial College London PhD student Laura Braun, and any discrepancies about which studies should be included were discussed and resolved.

Table 3.2 Flow chart detailing the classification process.

Start: Read the title		Code	
Is this paper a duplicate of another?	→ **Yes**	1	→ **Exclude**
↓ **No**			
Is the title about a non-human (or non-zoonotic) parasitic worm or a non-waterborne parasitic worm? (E.g. animal, plant, or insect parasite, free living species, or plant with a similar latin name)	→ **Yes**	2	→ **Exclude**
↓ **No**			
Is the title about the genetics or biochemistry of parasitic worms? (E.g. DNA screening, antigen formation, or protein interactions)	→ **Yes**	3	→ **Exclude**
↓ **No**			
Is the paper about the use of UV for detecting parasitic worms?	→ **Yes**	4	→ **Exclude**
↓ **No**			
Continue: Read the abstract			
Is the abstract about a non-human parasitic worm or a non-waterborne parasitic worm or the non-waterborne life stage (E.g. adult worm)	→ **Yes**	5	→ **Exclude**
↓ **No**			
Is the abstract primarily about the effects of a UV attenuated vaccine on the host animal? (E.g. the effects of the vaccine on antigen formation)	→ **Yes**	6	→ **Exclude**
↓ **No**			
Continue: Read the full paper			
Is the paper primarily about the effects of a UV attenuated vaccine on the host animal and/or infection?	→ **Yes**	7	→ **Exclude**
↓ **No**			
Does the paper contains significant errors (e.g. wavelength outside of UV range)	→ **Yes**	8	→ **Exclude**
↓ **No**			
Include the paper		9	

3.3.2 Data extraction

Where possible, the $\log_{10}$ reduction was calculated using the inactivation data presented in the studies and the equation

$$log_{10}\ reduction = log_{10}\frac{N_0}{N} \tag{3.1}$$

where N_0 is the proportion of viable organisms in the control sample and N is the proportion of viable organisms in the experimental sample. If the survival percentage of the control sample was not stated in a paper, it was assumed that 100% survived (except for studies assessing the worm burden). If 100% of the experimental sample was inactivated, it was assumed that one organism survived in order to calculate the minimum $\log_{10}$ reduction; if the study only reported the percentage of organisms, then it was assumed that 1% survived. The $\log_{10}$ reduction values were then interpolated to calculate the UV fluence required to achieve a 1- and 2-$\log_{10}$ reduction. If a 1-$\log_{10}$ reduction was not achieved in a study, then the data were extrapolated.

3.4 Results and discussion

In total 704 papers were returned by the search, resulting in 252 individual studies, once duplicates were removed. After classifying the papers by title and abstract, 59 studies were selected to be read in full, but two were unavailable. Forty-three studies from the search were ultimately included in the review and four additional studies that were referenced in the original papers were added, resulting in a total of 47 studies (Figure 3.1). Whilst 52 species of 23 genera were included in the search, results were returned for only 18 species of 10 genera (Table 3.3).
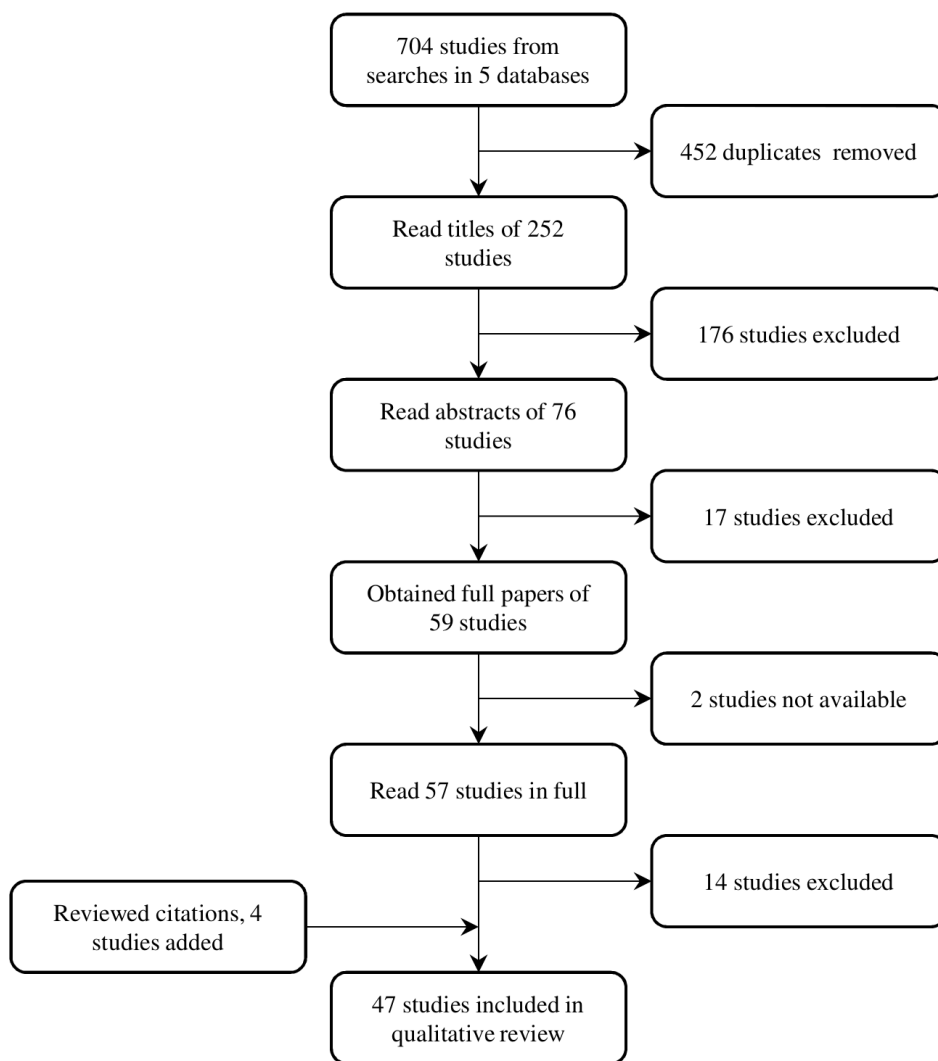
Figure 3.1 Preferred Reporting Items for Systematic reviews and Meta-Analyses classification flow diagram for ultraviolet sensitivity of helminths review.

Table 3.3 Summary of the genera included in this review, information on transmission route and prevention strategies from CDC (2019). One study (Nolf, 1932) examined two genera, *Trichuris* spp. and *Ascaris* spp., and is therefore listed twice.

Genus	NTD	# studies	Waterborne life stage	Transmission Route	Prevention
Ancylostoma	STH	3	Eggs	Skin contact with soil containing larvae.	Avoid walking bare foot. Reduce open defecation, effective sewage disposal.
Angiostrongylus	-	1	Third stage larvae	Food or water containing larvae including uncooked snails, slugs, or mollusk secretions.	Mainly food hygiene. Protect water from molluscs.
Ascaris	STH	16	Eggs	Food, water, or on hands contaminated with eggs passed in faeces.	Mainly handwashing and food hygiene. Reduce open defecation, effective sewage disposal.
Echinococcus	Echinococcosis	1	Eggs	Food, water, or on hands contaminated with eggs passed in dog faeces.	Mainly handwashing and food hygiene. Avoiding consumption of contaminated water.
Fasciola	FT	1	Miracidia, cercariae, metacercariae	Food or water contaminated with metacercariae.	Avoid consumption of raw water plants and contaminated water.
Hymenolepis	-	1	Eggs	Food, water, or on hands contaminated with eggs passed in faeces.	Mainly handwashing. Avoid consumption of contaminated food and water. Reduce open defecation, effective sewage disposal.
Opisthorchis	FT	1	Eggs, cercariae	Food containing metacercariae including undercooked fish.	Mainly food hygiene. Reduce open defecation, effective sewage disposal.
Schistosoma	Schistosomiasis	22	Miracidia, cercariae	Skin contact with water containing cercariae.	Mainly avoid contact with contaminated water. Reduce open defecation, effective sewage disposal.
Taenia	Taeniasis	1	Eggs, gravid proglottids	Food containing cysticeri including undercooked pork and beef.	Mainly food hygiene. Reduce open defecation, effective sewage disposal.
Trichuris	STH	1	Eggs	Food, water, or on hands contaminated with eggs passed in faeces.	Mainly handwashing and food hygiene. Reduce open defecation, effective sewage disposal.

3.4.1   Differences in experimental methods

3.4.1.1   Sources of ultraviolet light

Most studies used LP lamps but other sources include: sunlight, solar simulators, fluorescent lamps emitting in the UV-A and UV-B range, MP lamps emitting over a broad spectrum in the UV-C range, and monochromatic excimer lamps emitting in the UV-C range. It is difficult to directly compare studies that used sunlight or simulated sunlight with mercury arc lamps, as sunlight contains almost no radiation in the UV-C range. Studies using sunlight and long wavelength sources (UV-A and UV-B) have therefore been reviewed separately (Section 3.4.2) to studies using UV-C sources (Section 3.4.3). Where the source or wavelength was not stated in a paper it has been reviewed alongside the UV-C studies, as LP lamps are the most common.

3.4.1.2   Exposure procedure

Exposures were carried out in a number of different containers and the sample depth also varied between studies, from droplets on a glass cover slip to 25 mm deep samples in a culture dish (Ruelas *et al.*, 2007; Tromba, 1978). Different water matrices were also used for the exposures, for example deionized water, salt solutions, and filtered wastewater treatment plant effluent (Aladawi *et al.*, 2006; Chernicharo *et al.*, 2003; Heaselgrave and Kilvington, 2011; Lin *et al.*, 2011; Lipatov *et al.*, 2015; Menon and Bhopale, 1985; McGavock and Howard, 1980; Tian *et al.*, 2010).

Only one study used the Bolton and Linden (2003) protocol to calculate the fluence (Brownell and Nelson, 2006), therefore the fluences stated for all the other studies should be considered approximate at best. Some studies only recorded the exposure time and the fluence could not be calculated. Water depth and absorbance were not accounted for in most of the studies, and it is therefore likely that the reported fluences are over-estimations. In the case of samples being exposed in very small volumes of water (e.g. droplets on coverslips), this may cause the samples to dry out, and it is difficult to separate the effect of drying from the effect of UV light on the inactivation of the target organism. Similarly, some UV sources are known to produce a considerable amount of heat, and not all experiments controlled the temperature of the samples, which may have also contributed to inactivation of the target organism. Many microorganisms have the ability to reverse the damage caused by UV light through a variety of DNA repair mechanisms (2.4.2.3). However, the repair potential of helminths was explicitly examined in only one study (Ruelas *et al.*, 2007) and not all studies kept samples in the dark after UV exposure.

3.4.1.3   Viability assessment

A variety of methods were used to determine the viability of helminths following exposure to UV light, the most common was to assess the ability of eggs or larvae to reach the next stage of development inside an animal host (also known as *in vivo* methods). *In vitro* methods such as assessing the motility or morphology of larvae, and the ability of eggs to embryonate in culture dishes, were also used. The most appropriate method may vary between genera. The *in vivo* method is often seen as the most definitive way to establish viability although it may not always produce the most reliable fluence-response curves. This is because helminths have complex life cycles and often the number of organisms collected from a host is not directly proportional to the number of organisms in the inoculant. For example, in one

study there was a considerable difference in the number of mice that developed *Echinococcus granulosus* infections depending on whether they were inoculated with 500 or 2,000 eggs (0% and 75%, respectively) and in another study using *Taenia taeniaeformis* eggs the number of control organisms recovered from the host varied notably between experiments (4–30%), possibly as a result of incomplete recovery or because of an unknown underlying issue which caused a reduction or increase in the intensity of infection in some of the host animals (Williams and Colli, 1972; Konno *et al.*, 1997). Using the *in vivo* method is also likely to result in a higher level of inactivation than if an *in vitro* method is used to assess viability. This is because UV light inactivates pathogens by altering nucleic acids, and not all damage is immediately evident but can show up later in the development of the organism, as demonstrated in a number of the studies (Ariyo and Oyerinde, 1990; Dajem and Mostafa, 2007; Ghandour and Webbe, 1974; Ghandour and Webbe, 1975; Peng *et al.*, 2004; Tromba, 1978; Vieira and Rombert, 1974). Furthermore, as migration of eggs and larvae through the body can still pose a health risk, some authors suggest it is preferable to prevent entry to the bloodstream of the host and that it is necessary to demonstrate the organisms have been inactivated *in vitro* (Brownell and Nelson, 2006).

It is difficult to compare results between studies that use *in vivo* and *in vitro* methods. As the infection mechanism varies between genera, *in vitro* viability assessments may enable better comparisons, however further research is required to establish standardised methods for *in vitro* viability assessments of helminths. Selective dyes which stain only alive or dead cells may be suitable for this purpose and have previously been used to determine the viability of schistosome cercariae and schistosomula (Braun *et al.*, 2020a; Peak *et al.*, 2010). One study included in this review used methylene blue to identify dead schistosome cercariae which were stained violet blue, whilst live cercariae were left colourless (Zhang *et al.*, 2013).

3.4.2 Sunlight, solar simulators, and long wavelength artificial sources

Twelve studies investigated the effect of sunlight and long wavelength UV light (313 nm and 390 nm) on seven species of helminth: *Ancylostoma caninum, Ancylostoma ceylanicum, Angiostrongylus cantonensis, Ascaris suum, Ascaris lumbricoides, S. mansoni*, and *S. haematobium.* In sunlight experiments the eggs or larvae were exposed for a minimum of 15 minutes to over six hours of continuous sunlight. Some studies also investigated the effect of exposure to intermittent sunlight over a period of days. Much shorter time periods were required when using artificial UV-A and UV-B sources.

S. mansoni cercariae were the most sensitive to natural sunlight, requiring 60 minutes for all cercariae to be rendered motionless in one study, even on cloudy days (Krakower, 1940). Prah and James (1977) found *S. mansoni* and *S. haematobium* miracidia were equally sensitive to sunlight, however longer exposures were required than in studies using cercariae (Krakower, 1940). This suggests sensitivity to UV light may vary between different life-stages of the same species. Similarly, Spindler (1940) found single cell *A. suum* were more sensitive to sunlight than embryonated eggs (Table 3.4). *A. suum* was the most tolerant to sunlight; in one study single cell eggs were exposed to simulated sunlight between 290 nm and 800 nm at a fluence rate of $55\,\mathrm{mW/cm^2}$ for over six hours and only a 1.42-$\log_{10}$ reduction was achieved (Heaselgrave and Kilvington, 2011). However, it must be noted that a fluence rate over such a broad spectrum cannot be directly compared to a fluence rate in the UV range, as not all wavelengths have the same germicidal effectiveness. Furthermore, a high concentration of approximately 1 million eggs/mL was used and the study did not consider the effect of shielding,

where organisms higher in the water column may protect lower ones from UV exposure. The results of this study should therefore be considered conservative (i.e. under-estimate the true sensitivity of the eggs to UV light). Jones and Hollaender (1944) investigated the effect of simulated sunlight on *A. lumbricoides*, using a mercury source lamp which emitted light between $350\,nm$ and $490\,nm$ at a fluence rate of 0.1–$30\,mW/cm^2$. In this experiment the highest inactivation achieved was a 0.98-$\log_{10}$ reduction, but the authors noted that they would expect natural sunlight to be more damaging due to the presence of infrared radiation and higher temperatures. The samples were not mixed during the exposures; an effort was made to expose the eggs in single layers but there was an issue of "clumping" in some of the experiments.

Table 3.4 Sensitivity of some species to sunlight (interpolated data). Not all studies are shown because some contained insufficient information to calculate the $\log_{10}$ reduction.

Species	Conditions	Average exposure time (mins) to inactivate		References
		1-$\log_{10}$	2-$\log_{10}$	
A. caninum (larvae)	Direct sunlight, Korea	134	180	Roh, 1968
S. haematobium (miracidia)	Consistent sunshine, UK	196	-	Prah and James, 2009
A. suum (single cell)	Direct sunlight, Puerto Rico	91	125	Spindler, 1940
A. suum (embryonated)	Direct sunlight, Puerto Rico	300	311	Spindler, 1940
A. suum (single cell)	Solar simulator $55\,mW/cm^2$	317	-	Tromba, 1978

Two studies investigated the effect of intermittent sunlight on *A. lumbricoides*; both found that eggs were able to survive for much longer periods (up to 60 hours) than in other studies that used continuous sunlight (Miretski, 1956; Shikhobalova and Gorodilova, 1944). However, it should be noted that these experiments were carried out in Russia (at a high latitude) whereas other studies either used solar simulators or tropical sunlight containing higher levels of UV radiation (high fluence rates), which increases with proximity to the equator. Nenow (1960) confirmed that the germicidal effect of sunlight varies with altitude, suggesting that SODIS may be a more effective form of disinfection in communities located at higher altitudes, as shorter exposure times are required.

Of the hookworm species, no *A. caninum* larvae were able to survive 180 minutes exposure to sunlight (Roh, 1968), and only 60 seconds exposure to UV-A ($390\,nm$) light was required for larvae of *A. ceylanicum* to become visibly sluggish. After 30 minutes exposure, *A. ceylanicum* larvae began to lose motility completely and when hamsters were orally infected with a dose of 100 larvae, no worms were able to develop (Menon and Bhopale, 1985) (Table 3.5). Similarly, larvae of the nematode *A. cantonensis* exposed for 15 minutes to UV-A light were unable to develop inside an animal host (Kamath *et al.*, 1986). Only one study used UV-B light, which was shown to be relatively effective against *S. mansoni* miracidia. There were limited details on how the fluence was measured, but $86.1\,mJ/cm^2$ (approximately 2 minutes 30 seconds) was sufficient to achieve a 2-$\log_{10}$ reduction in the number of daughter sporocysts in snails, even though the miracidia did not appear harmed. When exposed to fluorescent white light, immediately after irradiation, the miracidia were able to photoreactivate, with significantly higher numbers of sporocysts in snails kept in light conditions than in snails kept in the dark (Ruelas *et al.*, 2007).

The eggs and larvae in these experiments were exposed to sunlight or long wavelength UV in small amounts of water, with no more than $3\,mL$ used in any of the experiments. However, SODIS is generally carried out in $2\,L$ bottles, which are laid on their side and left in direct sunlight. The depth of the water column will therefore be much higher than in the studies included in this review, increasing the amount of UV light that is absorbed by the water. It is therefore recommended that SODIS experiments are

Table 3.5 Sensitivity of some species to long wavelength ultraviolet radiation (interpolated data). Not all studies are shown because some contained insufficient information to calculate the $\log_{10}$ reduction.

Species	Fluence rate (mW/cm²)	Average exposure time (mins) to inactivate 1-$\log_{10}$	2-$\log_{10}$	References
S. mansoni (miracidia)	0.578	< 2	< 3	Ruelas *et al.*, 2007
A. cantonensis (larvae)	-	4	-	Kamath *et al.*, 1986
A. ceylanicum (larvae)	-	9	-	Menon and Bhopale, 1985

also carried out in the containers that will be used by households and local communities.

SODIS is currently mainly used for drinking water treatment, and 2 L bottles are therefore appropriate reactors as the treated water can be drunk straight from the bottle. However, some helminthiases can be transmitted via other routes related to insufficient hygiene provision or contact activities such as bathing and laundry which require larger amounts of water. Alternative reactors maybe required to effectively treat these volumes. Previous studies have used transparent plastic bags of various sizes as effective SODIS reactors although they have not been tested against helminths (Lawand *et al.*, 1997). These have the advantage of a higher surface area to depth ratio and the underside of the bag can be coated with a reflective surface to increase the reflection of UV light into the water (Dunlop *et al.*, 2011). A more recent yet very simple development is the use of 20 L transparent polypropylene buckets which have been shown to be effective SODIS reactors for drinking water (Polo-López *et al.*, 2019). These would be well suited for laundry and bathing but more research is required in this area.

3.4.3   Ultraviolet-C sources

Germicidal mercury arc lamps (253.7 nm unless stated otherwise) were used in 24 studies and an additional 12 studies used other UV-C light sources or did not specify the wavelength. There was a very large range in the inactivation data for *A. suum* and *A. lumbricoides*, with fluences from 11 to 3367 mJ/cm² required to achieve a 1-$\log_{10}$ reduction (Table 3.6). Only one study by Brownell and Nelson (2006) used the industry standard protocol to evaluate the fluence though Lucio-Forster *et al.* (2005) applied some factors, correcting for reflection, absorption, and divergence of the UV beam. The results of these two studies are reasonably similar with fluences of 100 and 84 mJ/cm² required to achieve 1-$\log_{10}$ inactivation of intact single cell *A. suum* eggs, respectively, although the difference is greater for a 2-$\log_{10}$ inactivation.

A study by Tromba (1978) suggested *A. suum* is more sensitive to UV light, achieving a 2.21-$\log_{10}$ reduction at 24 mJ/cm², even though the eggs used were in a later stage of development and other studies reported that tolerance to UV light increases with development stage (Nenow, 1960; Shalimov, 1935; Spindler, 1940). However, Tromba determined viability of the eggs by assessing the worm burden in animal hosts, which may have resulted in a higher level of inactivation than if an *in vitro* method was used, as not all damage is immediately evident (Brownell and Nelson, 2006; Nikulin, 1959; Stevens, 1909; Tromba, 1978). Peng *et al.* (2004) found that deformities began to show two weeks after single cell eggs were exposed to UV light (unknown wavelength) for 10–20 minutes, even though during the first week of incubation development of irradiated eggs matched that of the controls. Eggs that were exposed for less than 10 minutes appeared to develop normally for longer periods, with deformities showing only after three weeks. Chernicharo *et al.* (2003) used a prototype flow-through reactor to achieve a 0.77-$\log_{10}$ reduction in *A. lumbricoides* at 20.3 mJ/cm², but the eggs were dissected from

Table 3.6 Sensitivity of some species to ultraviolet-C radiation (interpolated data). Not all studies are shown because some contained insufficient information to calculate the $\log_{10}$ reduction.

Species	Average fluence (mJ/cm^2) to inactivate		References
	1-$\log_{10}$	2-$\log_{10}$	
S. japonicum (cercariae)	5	11	Tian et al., 2010
S. mansoni (cercariae)	6	9	Kumagai et al., 1992
S. mansoni (cercariae)	7	-	Dean et al., 1983
S. japonicum (cercariae)	8	16	Shi et al., 1990
S. mansoni (cercariae)	9	-	Kamiya et al., 1993
S. japonicum (cercariae)	10	19	Fang-Li et al., 1996
S. japonicum (cercariae)	11	21	Lin et al., 2011
S. japonicum (cercariae)	14	27	Wang et al., 1993
O. felineus (eggs)	27	-	Lipatov et al., 2015
A. suum (embryonated eggs)	11	22	Tromba, 1978
A. lumbricoides (single cell eggs)	> 26	-	Chernicharo et al., 2003
A. suum (single cell, decorticated)	30	56	Brownell and Nelson, 2006
A. suum (single cell eggs)	84	168	Lucio-Forster et al., 2005
A. suum (single cell eggs)	100	328	Brownell and Nelson, 2006
A. lumbricoides (single cell eggs)	3367	4748	Mun et al., 2009
T. taeniaeformis (eggs, decorticated)	10	20	Konno et al., 1997
T. taeniaeformis (eggs)	872	1300	Konno et al., 1997

Species	Average time (mins) to inactivate		References
	1-$\log_{10}$	2-$\log_{10}$	
S. mansoni (cercariae)	0.46	-	Ariyo and Oyerinde, 1990
S. mansoni (cercariae)	0.96	-	Ghandour and Webbe, 1975
S. mansoni (cercariae)	6	8.1	Standen and Fuller, 1959
F. gigantica (miracidia)	1.69	-	Riwidiharso and Billalodin, 2006
A. caninum (larvae)	11.78	-	Vieira and Rombert, 1974
H. diminuta (eggs)	16	-	McGavock and Howard, 1980

worm uteruses rather than collected from faeces or isolated from host intestines. It is possible that these eggs were more sensitive to UV light because the eggshells may not have fully developed (Brownell and Nelson, 2006). Furthermore, there was no information on how the fluence was calculated for the flow-through reactor.

One study compared the use of UV light and microwave radiation for disinfection of soil containing *A. lumbricoides* eggs, although experiments were also carried out in water. The authors found fluences over 3000 mJ/cm^2 were required to achieve any significant inactivation in water. Whilst some factors were applied during the fluence calculation, the collimating tube used in the experiments was 6 cm in diameter but only 10 cm long (Mun et al., 2009). In this region the beam from mercury arc lamps is divergent, and radiometers can produce errors if they are used to measure the irradiance very close to the source. It is generally recommended that a collimating tube four times as long as the diameter is used with mercury arc lamps (Bolton and Linden, 2003). It is therefore possible that the fluence was actually less than was stated in the paper.

Another study suggested that exposure to UV light actively increased the larval development of *A. lumbricoides*, even when exposed to fluences greater than 15 000 mJ/cm^2 (Aladawi et al., 2006). This contradicts the results of all the other studies included in the review and goes against the general understanding of the effect of UV light on microorganisms and UV disinfection. As with the previous study the irradiance was measured only 5 cm from the source and it is unclear if a collimating tube was used at all. The number of eggs in each sample was not specified and it is unclear if the suspensions were stirred. Furthermore, eggs were suspended in filtered secondary wastewater effluence, which may

have absorbed a considerable amount of UV light, for which there was no correction applied. However, some inactivation would still have been expected. It is possible that some repair occurred (it is unclear if the samples were kept in the dark following exposure) or the accelerated development may be a result of the increase in temperature, which was not measured during the experiments, and has previously been shown to increase the rate of development in *Ascaris* spp. (Aladawi *et al.*, 2006).

An early study by Nolf (1932) compared two species of soil-transmitted helminth, and found that *Trichuris trichiura* eggs were less sensitive to UV light (unknown wavelength) than *A. lumbricoides* eggs. Non-standard units were used to measure the extent of the exposure to UV light which means this paper cannot be compared to other studies, and there have been no further studies using *Trichuris* spp. support this. The hookworm *A. caninum* appeared to be the most sensitive soil-transmitted helminth studied, however experiments were carried out using larvae, not eggs, and the fluence was not recorded experiments. The initial $\log_{10}$ reductions achieved were relatively low, only 0.38 after five minutes exposure, however exposed larvae were not able to survive for as long as controls. Larvae exposed for five minutes did not live more than five days, whereas 52% of larvae exposed for 30 seconds were able to survive five days or more (Vieira and Rombert, 1974).

Taenia taeniaeformis eggs were very tolerant to UV light, requiring $720\,\mathrm{mJ/cm^2}$ to achieve a 0.65-$\log_{10}$ reduction in the number of cysts recovered from the host when compared to control eggs (Konno *et al.*, 1997). However, only one study investigated *Taenia* spp. and there are few details of how the fluence was calculated. Furthermore, there was a notable difference in the number of cysts recovered from the controls in each of the experiments (4–30%) and it is unclear why this occurred. In the same study only $30\,\mathrm{mJ/cm^2}$ was required for 3-$\log_{10}$ reduction when the embryophore had been removed, suggesting that as with the eggshell for *Ascaris* spp., the embryophore is key to *Taenia* spp. tolerance to UV light (Brownell and Nelson, 2006; Konno *et al.*, 1997; Lucio-Forster *et al.*, 2005).

The importance of the eggshell is less clear for other helminths in this review due to the lack of studies. A flow-through reactor with excimer lamps at 222 nm and 282 nm achieved a 0.92-$\log_{10}$ reduction in *Opisthorchis felineus* eggs in wastewater at a fluence of $25\,\mathrm{mJ/cm^2}$, suggesting it is relatively sensitive to UV light (Lipatov *et al.*, 2015). However, a very small sample size was used, and it is unclear how the fluence was determined. Only two experiment samples were tested and the number of eggs in the samples prior to UV exposure was not known, only one control sample was tested to calculate the $\log_{10}$ reductions. *Hymenolepis diminuta* ova that were exposed to UV light for a minimum of 30 minutes were unable to develop into cysts. At 15 minutes exposure one cyst was able to develop, though this was deformed (McGavock and Howard, 1980). No infections developed in mice injected with 500 exposed *Echinococcus granulosus* eggs that had been exposed to UV light (unknown wavelength) for 24 hours. Yet, when mice were injected with 2000 exposed eggs, 75% were able to develop infections, although significantly fewer eggs were able to develop into cysts than in control mice (0.15% compared to 0.7%). This was probably due to the proportion of viable embryos in each of the doses (Williams and Colli, 1972).

Of the trematodes, only one study used miracidia of *Fasciola gigantica*. There was a significant reduction in cercariae shed from snails when they were infected with one exposed miracidium, compared to one control miracidium, even at very short exposure times less than 70 seconds (Riwidiharso and Billalodin, 2006). The effect of UV light on *Schistosoma* spp. has been the most widely studied. The majority of papers were immunisation studies, investigating the use of UV-attenuated cercariae

to produce a vaccine against human schistosomiasis. In these experiments cercariae were exposed to a fluence high enough to cause damage and prevent development into adult worms, but that still allowed penetration of the host's skin. The focus was therefore on the worm burden, and details of the exposure methods were often limited. In the immunisation papers a 1-$\log_{10}$ reduction in worm burden was achieved with fluences of 5–14 mJ/cm^2 or exposure times of less than one minute (Ariyo and Oyerinde, 1990; Dean *et al.*, 1983; Fang-Li *et al.*, 1996; Kamiya *et al.*, 1993; Kumagai *et al.*, 1992; Lin *et al.*, 2011; Ruppel *et al.*, 1990; Shin *et al.*, 2001; Tian *et al.*, 2010; Wang *et al.*, 1993; Zhang *et al.*, 2013). The direct effect of UV light on cercariae was first studied by Krakower (1940) who found 45 minutes exposure to a mercury source lamp (unknown wavelength) was required to kill the whole sample. Shorter exposures were still able to cause damage, making the cercariae less motile than the control samples, though they were able to recover from their injuries within 30 minutes and survived for as long as the controls, suggesting schistosome cercariae have some repair potential. Standen and Fuller (1959) found that only four minutes was required to kill 100% of *S. mansoni* cercariae in their study, but the LP lamp used was very near to the sample (2 cm) and it is unclear if the authors controlled the water temperature. Older mercury arc lamps are known to have produced a lot of heat and cercariae are inactivated within minutes at 45 °C and almost instantly at temperatures above 50 °C (Braun *et al.*, 2018).

Ghandour and Webbe (1975) studied the effect of UV light on the ability of *S. mansoni* and *S. haematobium* cercariae to penetrate skin. There was a significant increase in mortality during skin penetration when cercariae were exposed for 5–20 seconds, even though they did not appear to be harmed. 10–11% of exposed cercariae were unable to penetrate at all compared to 2–3% of the control sample (Ghandour and Webbe, 1974; Ghandour and Webbe, 1975). Another study found that short exposure times caused a reduction in the motility of cercariae, but this only became apparent four hours after exposure (Ariyo and Oyerinde, 1990). Cercariae penetrate skin through enzyme activity and mechanical action, a combination of motility reduction and inhibition of enzymes may have prevented cercarial penetration.

Two studies used scanning electron microscopy (SEM) to examine the physical damage caused by UV light to *S. mansoni*. Mohammed (1999) showed that adult worms developed from irradiated cercariae had lost their spikes and suffered from torn tubercles and lesions, causing sexual anomalies and sterility, possibly explaining the reduction in fecundity of worms derived from irradiated cercariae in other studies (Ariyo and Oyerinde, 1990; Dean *et al.*, 1983). Later Dajem and Mostafa (2007) used SEM to examine the damage on the surface of cercariae and discovered that irradiated samples appeared to be physically the same as control cercariae, suggesting the damage observed in adult worms is either a result of mutagenic effects of UV light which only appear later in development, or as a result of the hosts immune response to irradiated cercariae. Another study found that UV exposure modified the structure of molecules on the surface of *S. mansoni* cercariae, even though no morphological changes occurred. This may have caused an enhanced immune response by the host (Kusel *et al.*, 1989).

Significantly more male *S. mansoni* worms were able to develop from irradiated cercariae in one study, suggesting that males may be more tolerant to UV light than females (Rombert and Vieira, 1977). This also may explain the reduction in fecundity observed in other studies, but further research is required to confirm this (Ariyo and Oyerinde, 1990; Dean *et al.*, 1983). *S. mansoni* and *S. japonicum* were shown to be equally sensitive to UV light, suggesting the inactivation mechanism is the same in

both species (Ruppel *et al.*, 1990). Only one study used *S. haematobium* cercariae, which were found to be slightly more tolerant that *S. mansoni* cercariae, however no statistical analysis was performed (Ghandour and Webbe, 1975). Prah and James (1977) found there was no difference in the response of *S. mansoni* and *S. haematobium* miracidia to UV light from LP lamps.

3.4.4 Impact of the water matrix

Whilst many different suspension media have been used, only one study eligible for inclusion in the review investigated the impact of turbidity or the water matrix on UV disinfection. Prah and James (1977) found a 15.4% reduction in the rate of movement of miracidia was observed when using 1% turbid water, compared to a 60.3% reduction when distilled water was used. However, it should also be noted that distilled water has been shown to kill schistosome cercariae, and it may have a similar effect on miracidia (Frick and Hillyer, 1966). If UV or solar disinfection is to be used effectively for household and community scale water treatment this aspect requires further research, preferably using water samples collected from the environment. If water collected from local waterbodies is of particularly poor quality (e.g. high turbidity, iron, or organic matter content), consideration may need to be given to pre-treatment, such as filtration or sedimentation.

3.5 Conclusions

Compared to bacterial, viral, and protozoan pathogens there has been little research into the effectiveness of UV light at inactivating helminth eggs or larvae and the disinfection mechanisms involved, even though these pathogens are endemic in many low-income countries where UV disinfection and SODIS are promoted as sustainable forms of household and community scale water treatment (Section 2.4.7). The majority of the studies in this review investigated the effect of UV light on either *Schistosoma* spp. or *Ascaris* spp., and many were immunisation studies used for developing UV-attenuated vaccines, with a focus on the host response to irradiated larvae or eggs, rather than complete inactivation of the target organism or applications to water treatment.

There were limitations to almost all of the UV disinfection studies, the most significant being the lack of a standardised procedure for calculating the UV fluence to which samples were exposed – 68% of studies were carried out before the industry standard protocol for fluence measurement was published in 2003 (Bolton and Linden). In the SODIS studies, experiments were carried out using very small amounts of water which is not representative of how disinfection will take place in practice. Very few studies considered the impact of water quality or accounted for the absorbance of UV by the water column or the effect of shielding caused by suspended particles and other organisms. Mercury arc lamps are known to produce considerable amounts of heat and it is not clear which of the studies controlled the water temperature. In some studies the fluence was not recorded at all and only one paper investigated the repair potential. The methods for determining the viability of larvae or eggs varied, even between papers using the same genera, and this resulted in large ranges in the fluence response, most notably for *Ascaris* spp. Furthermore, the survival percentage of control samples was not stated in all studies and assumptions were made to calculate the log reductions presented in this review.

These limitations make it difficult to directly compare the studies, however some conclusions can be

drawn. All helminths included in this review could be inactivated by UV light at certain fluences and wavelengths, but the number of species studied was limited and the data are insufficient to produce detailed recommendations for household or community scale UV or solar disinfection of water in endemic regions. Whilst the viability methods varied, helminths which hatch in the environment were generally more sensitive to UV light than species which stayed in the egg until after they had infected the host. Studies found that eggs were much more sensitive to UV when the shell or embryophore had been removed, suggesting they play a key role in the tolerance to UV light for some species. Relatively low fluences (5–$14\,\mathrm{mJ/cm^2}$ at $253.7\,\mathrm{nm}$) were required to prevent schistosome cercariae from developing into adult worms in animal hosts and achieve a 1-$\log_{10}$ reduction in worm burden. However the direct effect of UV light on the cercariae was not quantified. At these fluences cercariae were visibly unharmed and were still able to actively find and infect a host (penetrate the skin), but they were not able to develop into adult worms.

This systematic review has demonstrated that evidence exists to suggest that UV disinfection may be effective against schistosome cercariae and other helminths, but the data are insufficient to produce detailed recommendations for household or community scale UV or solar disinfection of water in endemic regions. To aid the design of these water treatment systems the following research priorities have been identified:

1. The industry standard protocol should be used to accurately determine the UV fluence in experiments using mercury arc lamps to ensure the results are comparable and repeatable, taking into consideration the potential for photoreactivation of the target organisms. There is currently no standard protocol for determining the UV fluence from UV LEDs. Until this has been agreed by the UV industry researchers should stay up-to-date with developments in this area and studies using these devices should include a detailed methods section, clearly stating the type of device and protocol used.

2. Where possible, *in vitro* methods for determining viability of helminths should be prioritised, as these allow for better comparisons between genera and are not reliant on animal testing.

3. Helminths which hatch from their eggs in the environment should be prioritized for UV disinfection studies, as larval forms may be more sensitive to UV light than helminth eggs.

4. SODIS experiments should be carried out in countries where the helminths are endemic, using appropriate containers and storage times, to determine if current SODIS guidelines are sufficient for helminth inactivation.

5. Experiments should be carried out in lab conditions to determine the fluence-response relationship of the target organism, and in field conditions to fully understand the effectiveness of UV disinfection in real water matrices. Water samples and helminth eggs or larvae collected from the environment should be used and recommendations made regarding the turbidity and UV absorbance limits at which UV and solar disinfection can be used for helminth inactivation.

4 Ultraviolet disinfection of *Schistosoma mansoni* cercariae at four peak wavelengths

4.1 Abstract

Schistosomiasis is a parasitic disease that is transmitted by skin contact with waterborne schistosome cercariae. Mass drug administration with praziquantel is an effective control method, but it cannot prevent reinfection if contact with cercariae infested water continues. Providing safe water for contact activities such as laundry and bathing can help to reduce transmission. The direct effect of UV light on *S. mansoni* cercariae was investigated using UV LEDs and a LP lamp. *S. mansoni* cercariae were exposed to UV light at four peak wavelengths: 255 nm, 265 nm, 285 nm (UV LEDs), and 253.7 nm (LP lamp) using bench scale collimated beam apparatus. The UV fluence ranged from 0–300 mJ/cm^2 at each wavelength. Cercariae were studied under a stereo-microscope at 0, 60, and 180 minutes post-exposure and their motility and morphology were assessed to determine if they were alive or dead. Very high UV fluences were required to kill *S. mansoni* cercariae, when compared to most other waterborne pathogens. At 265 nm a fluence of 247 mJ/cm^2 (95% confidence interval (CI): 234–261 mJ/cm^2) was required to achieve a 1-log$_{10}$ reduction at 0 minutes post-exposure. Cercariae were visibly damaged at lower fluences, and the log$_{10}$ reduction increased with time post-exposure at all wavelengths. Fluences of 125 mJ/cm^2 (95% CI: 111–146 mJ/cm^2) and 99 mJ/cm^2 (95% CI: 85–113 mJ/cm^2) were required to achieve a 1-log$_{10}$ reduction at 60 and 180 minutes post-exposure at 265 nm. At 0 minutes post-exposure 285 nm was slightly less effective, but there was no statistical difference between 265 nm and 285 nm after 60 minutes. The least effective wavelengths were 255 nm and 253.7 nm. Due to the high fluences required, UV disinfection is unlikely to be an energy- or cost-efficient water treatment method against schistosome cercariae when compared to other methods such as chlorination, unless it can be demonstrated that UV-damaged cercariae are non-infective using alternative assay methods or there are improvements in UV LED technology.

4.2 Introduction

Improvements in WASH infrastructure are required for sustainable control and elimination of schistosomiasis as a public health problem (Secor, 2014; Campbell *et al.*, 2014). Treating water that has been collected or abstracted from contaminated freshwater bodies before using it for contact activities could provide a safe source of water that is not reliant on significant developments in regional infrastructure. Solar-powered UV disinfection has been shown to be a sustainable and effective form of household and community scale water treatment in a number of low-income countries (Opryszko *et al.*, 2013; Hunter *et al.*, 2013; Reygadas *et al.*, 2018). It does not require chemicals, does not form disinfection byproducts, and has no harmful effects from overdosing. UV LEDs have the additional benefits over conventional mercury lamps of not containing toxic mercury, lower power requirements, and longer lifespans (Chen *et al.*, 2017). They are also durable and small so they can be fitted into pipes or water pumps (Linden *et al.*, 2019; Sundar and Kanmani, 2020). The cost of germicidal UV LEDs may be

prohibitive for their use in WASH programmes at the moment, however it is predicted they will be commercially available for less than 1 USD/W by 2030 (Krames, 2020).

Previous studies have assessed the viability of UV exposed schistosome cercariae by using animal testing. Based on the systematic review in the previous chapter, a fluence of 5–$14\,\mathrm{mJ/cm^2}$ at 253.7 nm was required to achieve a 1-$\log_{10}$ reduction in worm burden in animal hosts. However the direct effect on cercariae was not recorded and at these fluences cercariae were still able to penetrate the skin. Furthermore, the methods for determining the UV fluence were not clear and not in accordance with the widely accepted Bolton and Linden (2003) protocol. The fluences should therefore be considered approximate at best. Some studies did examine the direct effect of UV light on schistosome cercariae, demonstrating that short exposures to UV light reduced motility and skin penetration (Standen and Fuller, 1959; Ghandour and Webbe, 1975; Ariyo and Oyerinde, 1990), but the fluence rate was not recorded in any of these studies and the experiments cannot be reproduced.

Using animal testing to assess worm burdens may not provide the most reliable fluence-response curves for helminths due to incomplete recovery of worms and fundamental differences in infection of animal and human hosts (Cheever, 1969; Brownell and Nelson, 2006; Abdul-Ghani and Hassan, 2010). Alternative methods for assessing inactivation include using skin samples to examine infectivity or studying the morphology and motility of cercariae under the microscope to determine if they are alive or dead (Table 4.1). Furthermore, skin penetration by cercariae can cause cercarial dermatitis and migration of the parasite around the body before it fully develops into an adult worm may result in Katayama syndrome in sufferers of acute schistosomiasis. Symptoms of Katayama syndrome include sudden fever, headaches, muscle pain, abdominal tenderness, malaise, and fatigue (Ross *et al.*, 2007; Langenberg *et al.*, 2019). Therefore, to make water safe from schistosome cercariae, treatment processes should aim to prevent cercariae from entering the bloodstream or prevent skin penetration entirely.

Table 4.1 Possible methods for assessing inactivation of ultraviolet exposed schistosome cercariae.

Measure	Definition	Method
Non-viable	Cercariae are alive and able to penetrate skin but they are unable to develop into adult worms	Infect animal hosts with cercariae and assess the number of adult worms that are able to develop
Non-infective	Cercariae are alive but are unable to attach to or penetrate skin	Expose cercariae to human skin samples and count the number of cercariae present in the water before and after exposure to skin
Dead	Cercariae are dead	Examine morphology and motility of cercariae under the microscope

In this chapter the direct effect of UV disinfection on *S. mansoni* cercariae is examined by studying the morphology and motility of cercariae post-UV exposure to determine if they are alive or dead. Experiments are conducted with a LP lamp and UV LEDs at three peak wavelengths: 255 nm, 265 nm, and 285 nm, using previously published protocols for fluence determination (Bolton and Linden, 2003; Kheyrandish *et al.*, 2018). The visible damage to *S. mansoni* cercariae caused by UV exposure is discussed and the fluence-response at each wavelength is established.

4.3   Methods

4.3.1   Ethics statement

The Wellcome Sanger Institute (UK) maintains the complete life cycle of *S. mansoni* NMRI (Puerto Rican) strain. All the regulated procedures were conducted under the Home Office Project License No. P77E8A062, and protocols were revised and approved by the Animal Welfare and Ethical Review Body (AWERB) of the Wellcome Sanger Institute. The AWERB is constituted as required by the UK Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012.

4.3.2   Snail maintenance

Biomphalaria glabrata snails (Puerto Rican strain) were bred from an existing collection at the Wolfson Wellcome Biomedical Laboratories at the Natural History Museum. Snails were kept in 2–3 L of bottled spring water (Volvic, pH 7) in shallow plastic trays, covered with a glass plate or loose fitting plastic lid. A maximum of 25 snails were kept in each aquarium, to avoid overcrowding. The room temperature was maintained at 27 °C and the lighting was timer-controlled to provide 14 hours of light and 10 hours of darkness, mimicking natural conditions. Snails were fed dried lettuce and fish flakes twice weekly. Deterioration in water quality can have a dramatic and sudden impact on snails, and may result in death. Therefore the aquaria were inspected daily, and dead snails, which can harbour bacteria and other contaminants, were immediately removed using forceps. The water was changed at least once every three weeks and the aquaria were disinfected by filling them with distilled water and microwaving for 2 minutes. The aquaria were dried and allowed to cool before refilling with bottled spring water.

Rotifers (*Rotaria rotatoria*), microscopic commensals that live on snail shells, are common contaminants in laboratory-maintained snail colonies (Lewis *et al.*, 1986). They produce a compound which has a paralytic effect on schistosome cercariae (although this is reversed if cercariae are placed into clean water) and are known to inhibit cercarial production (Stirewalt and Lewis, 1981; Gardner *et al.*, 2021). Rotifers reproduce asexually and a colony can be established from a single organism, making it difficult to completely eradicate them, but numbers can be controlled by regularly cleaning the snail shells. Snails were inspected under the stereo microscope weekly, if rotifers were present the snail shells were cleaned with a soft-tipped paint brush then rinsed in bottled spring water. Snails were inspected again under the microscope to check for remaining rotifers, then they were returned to a clean aquarium.

4.3.2.1   Snail breeding

Aquaria were inspected daily for snail egg production. Egg masses that had been deposited on the side and bottom of the aquaria were carefully removed with a knife and transferred to a beaker containing 30 mL of 1% bleach solution (Sainsbury's Basics bleach, < 5% NaClO, diluted in bottled spring water) for 10 minutes, to inactivate pathogens and rotifers. Disinfected eggs were transferred to a beaker containing artificial spring water at 27 °C and covered with a glass plate. Eggs hatched after 3–4 days. Newly hatched snails were kept in the beaker and fed powdered fish flakes for approximately three weeks, before being transferred to an aquarium.

(a)   **(b)**
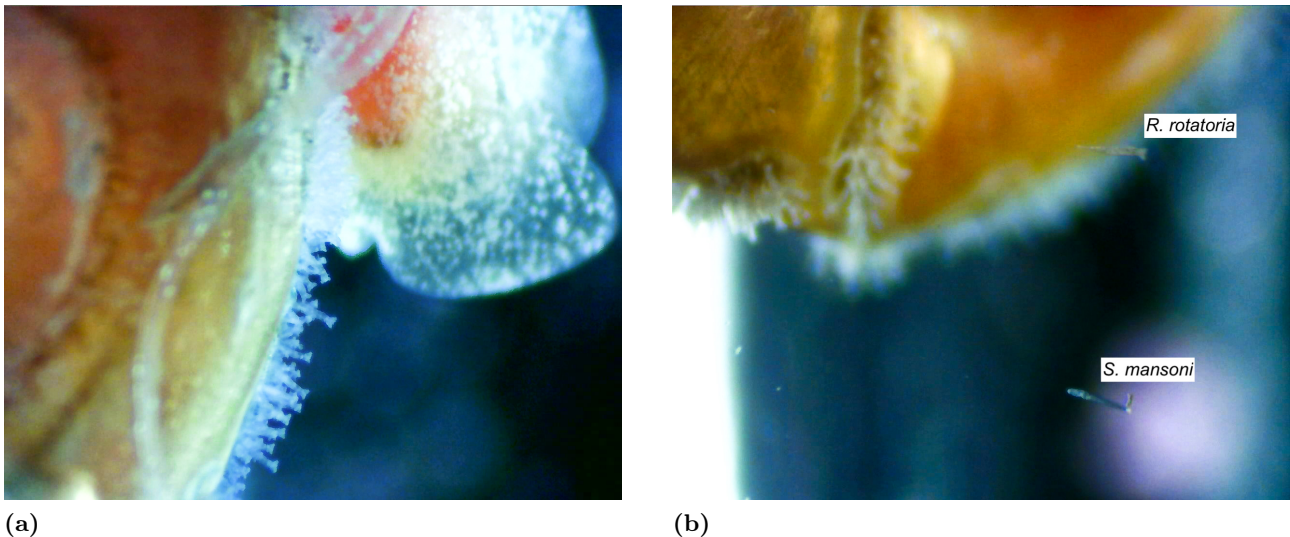
Figure 4.1 (a) A snail shell infested with rotifers *Rotaria rotatoria* and **(b)** a free swimming rotifer compared to a *Schistosoma mansoni* cercariae.

4.3.2.2   Snail infection

Snail infections were carried out with *S. mansoni* from infected mouse livers, obtained monthly from the Wellcome Sanger Institute. Mice were infected with 250 cercariae and the livers were harvested 40 days post-infection. Livers were scraped through an 850 µm sieve to break-up the tissue and the sieve was rinsed with bottled spring water. The resulting tissue mixture was passed through a 40 µm Pitchford funnel to extract the eggs, which were concentrated in the bottom of the funnel (Pitchford and Visser, 1975). The egg mixture was transferred into a Petri dish and exposed to bright light for 15 minutes, to allow miracidia to hatch from the eggs. Thirty to fifty *B. glabrata* snails that were six to ten weeks old (or <1 cm diameter) were selected for infection. To ensure even exposure, snails were individually placed into separate beakers, each containing 20 mL of bottled spring water and 5 miracidia, that were collected using a 10 µm pipette. Snails were exposed for 12 hours, before being transferred to a clean aquarium. Cercariae production commenced 25–30 days post-infection.

Snail breeding and infection was supported by Dr Fiona Allan and Dr Aidan Emery, WISER collaborators from the Natural History Museum. Preliminary UV experiments were carried out at the Natural History Museum, however due to the Covid-19 pandemic, experiments were moved to the Roger Perry Laboratory (RPL) at ICL in June 2020. During this time, all snail breading and infections were carried out by Dr Allan and Dr Emery and snails were transferred to the RPL in the prepatent period. Snails were kept in an incubator at 27 °C and maintained as described in Section 4.3.2. Egg-masses were removed from the aquaria, allowed to dry out completely (i.e. killed) and discarded, as the facilities at the RPL are not suitable for breeding snails.

4.3.3   Preparation of cercariae and enumeration

Snails were kept in the dark for 24 hours prior to all experiments, to enable maximal shedding of cercariae. Five to ten infected snails were selected at random and rinsed in bottled water. Snails were transferred to a beaker containing 5–8 mL of bottled spring water (Volvic, pH 7, 27 °C) and placed under bright, visible light for 60–90 minutes, to induce shedding of cercariae. After this time, the snails

were removed and the cercariae infested water was filtered through a 200 µm polyester mesh to remove snail faeces. Three 100 µL aliquots were taken by pipette and 10 µL of Lugol's iodine was added to fix and stain the cercariae so they could be easily counted. Based on the mean concentration, the required volume of infested water to achieve 100–120 cercariae per sample (typically 200 µL of cercariae infested water per sample, maximum 400 µL) was added to clean bottled spring water in glass Petri dishes to achieve a final sample volume of 3.8 mL. Fresh cercariae were shed daily for each set of UV exposures and all exposures were completed within a maximum of six hours of the cercariae having been shed from a snail.

4.3.4 Irradiance measurement and fluence determination

Cercarial suspensions were exposed to polychromatic UV light at three peak wavelengths, 255 nm, 265 nm, and 285 nm, using a Triple Wavelength PearlLab Beam UV LED Device (Aquisense Technologies, Kentucky, USA). The device is equipped with an array of nine LEDs (three at each wavelength), power control, thermal management, and a 76 mm internal diameter optimising tube. The LEDs are arranged in an evenly space ring, and each wavelength can be operated independently or simultaneously. This device was chosen for the experiments because it produces a highly uniform irradiance distribution and has been designed and tested specifically for fluence-response experiments (Beck *et al.*, 2017; Green *et al.*, 2018; Hull and Linden, 2018; Sholtes *et al.*, 2019; Sholtes and Linden, 2019; Woo *et al.*, 2019; Oliveira *et al.*, 2020). For comparison, cercariae were also exposed to near monochromatic radiation from a LP lamp, emitting at 253.7 nm, set up as a collimated beam. A schematic diagram showing the set up of each system is showing in Figure 4.2. The spectral power distribution of the UV LEDs and the LP lamp and the technical specifications of the LEDs are shown in Figure 4.3.

The irradiance was measured using radiometry and the average fluence rate inside the sample was calculated based on the methods determined by Kheyrandish *et al.* (2018) (for the UV LEDs) and Bolton and Linden (2003) (for the LP lamp), as described below.
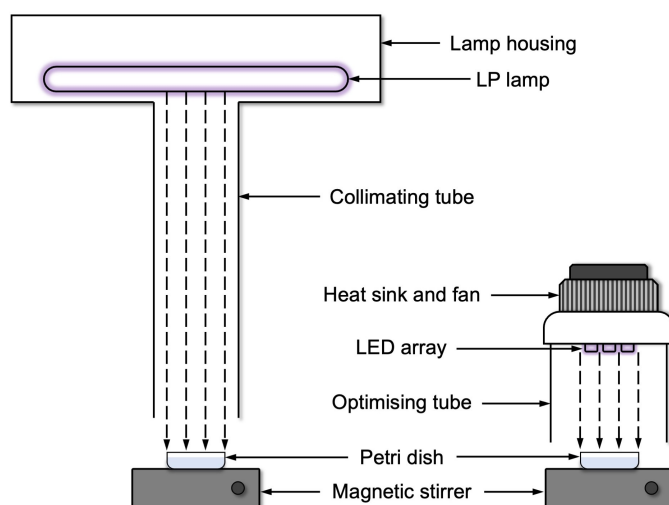
Figure 4.2 Schematic diagram showing the arrangement of the low pressure mercury arc lamp set up as a collimated beam and the ultraviolet light-emitting diode device (not to scale).
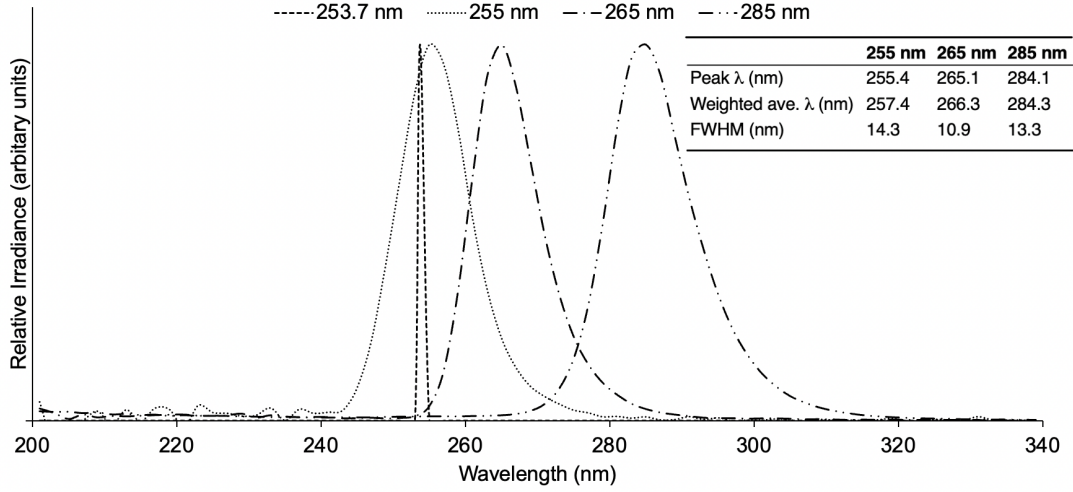
Figure 4.3 Spectral power distribution for the ultraviolet light-emitting diodes and low pressure mercury arc lamp and technical specifications of the ultraviolet light-emitting diodes. Note that the graph displays the relative irradiance, the actual optical power output of each source is not equal.

4.3.4.1 Radiometry

The incident irradiance at the sample surface was measured using an ILT 1700 radiometer equipped with a SED 270 detector (both International Light Technologies, Massachusetts, USA, calibrated annually). All UV LED sources were switched on at least 15 minutes before measuring the irradiance to allow the outputs to stabilise, the LP lamp was switched on 30 minutes before measurement.

UV LEDs   The radiometer detector was placed beneath the PearlLab Beam unit such that the surface of the detector was 10.4 cm away from the UV LEDs. The detector was moved in 5 mm increments across a 30 mm×30 mm Cartesian grid that was centred beneath the UV LEDs, and the incident irradiance measurement at each location was noted (Figure 4.4a). Irradiance measurements were then interpolated to a polar gird with the spacing of 1° and 1 mm, on a 29 mm diameter planar that was equal to the average internal diameter of the glass Petri dishes that were to be used in the experiments (Figure 4.4b). The interpolated values were then weighted by the area of each grid location and the weighted average incident irradiance across the surface of the Petri dish, $\overline{E^0}$, was calculated using the equation by Kheyrandish *et al.* (2018)

$$\overline{E^0} = \frac{\sum_{i=1}^n w_i E^0 \left(r, \theta\right)_i}{\sum_{i=1}^n w_i} \tag{4.1}$$

where $E^0 \left(r, \theta\right)_i$ is the interpolated incident irradiance measurement at the polar grid location $\left(r, \theta\right)_i$ and n is the total number of grid locations. w_i is the weighted area of each grid location $w_i = \frac{A(r,\theta)}{A}$, where $A \left(r, \theta\right)$ is the area of the polar grid location and A is the surface area of the Petri dish.

To account for the variation in the sensitivity of the radiometer detector over the spectral power distribution of the polychromatic UV LEDs, a sensor factor (SF) was applied to the weighted average incident irradiance. The following equation was adapted from Bolton and Linden (2003)

$$SF = \frac{1}{\sum_\lambda N_\lambda \times S_\lambda} \tag{4.2}$$

80
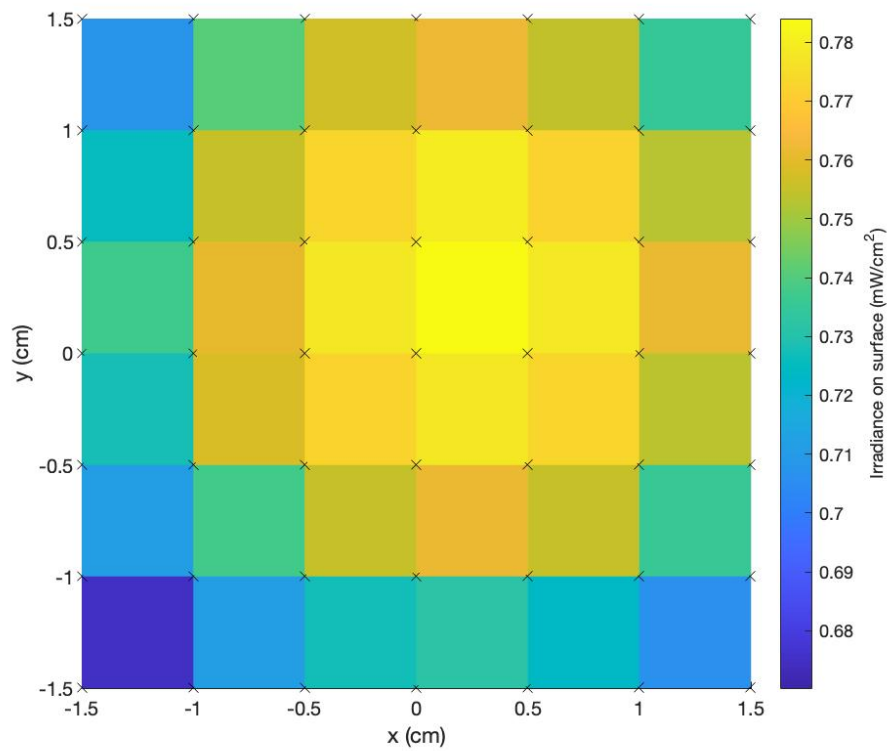
(a)
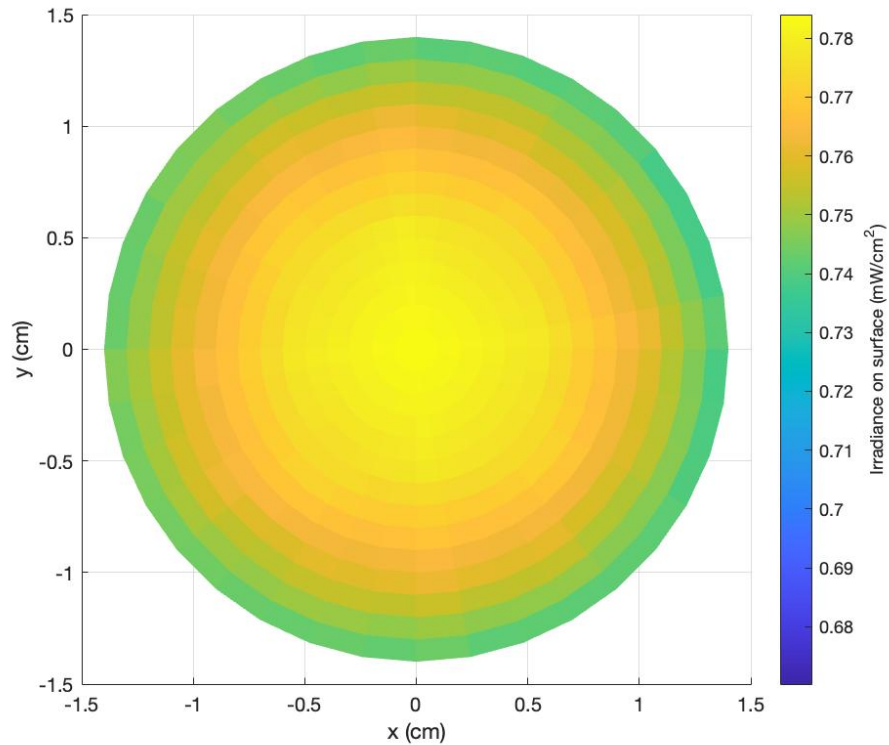
(b)

Figure 4.4 Example irradiance surface plots of 285 nm ultraviolet light-emitting diodes at 10.4 cm from source **(a)** measured on a Cartesian grid (× denotes measurement location) and **(b)** interpolated to a polar grid equal to the Petri dish diameter.

where N_λ is relative lamp emission at wavelength λ (normalised to unity) and S_λ is the detector sensitivity at wavelength λ relative to the peak sensitivity.

The uniformity of the irradiance distribution across the surface of the Petri dish was evaluated by calculating the corresponding coefficient of variation (CV), as proposed by Kheyrandish *et al.* (2018)

$$CV = \frac{\sqrt{\frac{\sum_{i=1}^{n} w_i \left(E^0(r,\theta)_i - \overline{E^0} \right)^2}{\sum_{i=1}^{n} w_i}}}{\overline{E^0}} \tag{4.3}$$

LP lamp The radiometer detector was placed beneath the collimated beam so that the surface of the detector was 31.5 cm away from the UV source. In accordance with Bolton and Linden (2003) it is only necessary to measure the incident irradiance was measured at 5 mm increments along the orthogonal axis of the sample surface for LP lamps. The irradiance distribution across the surface of the Petri dish can then be estimated by assuming the irradiance at point (x, y) as the geometric average of the irradiances at point $(x, 0)$ and $(0, y)$. The Petri factor (PF) is defined as the ratio of the average irradiance $\left(\overline{E^0(x,y)} \right)$ across the Petri dish surface to the irradiance at the centre of the dish $\left(E^0(0,0) \right)$ (Bolton and Linden, 2003)

$$PF = \frac{\overline{E^0(x,y)}}{E^0(0,0)} \tag{4.4}$$

For the LEDs a CV less than 6.7% indicates a uniform irradiance distribution over the surface of the sample and is equivalent to a Petri factor greater than 0.9 for a LP lamp with collimated beam (Kheyrandish *et al.*, 2018). CV values ranged from 2.0% to 2.4% for all UV LED exposures, and Petri factors ranged from 0.9813 to 0.9942 for all LP lamp exposures. Irradiance measurements were repeated following the completion of all UV exposures, to confirm they were within 5% of the initial measurement (US EPA, 2006). To determine the average fluence rate in the sample, factors were applied to incident irradiance measurements to correct for the absorbance (water factor), divergence, and reflection of UV light (Bolton and Linden, 2003).

Water factor (WF) A UV-2401PC spectrophotometer (Shimadzu, UK) was used to measure the absorbance of the sample at the weighted average wavelength of the UV LEDs and the peak wavelength of the LP lamp and the WF was calculated using the equation

$$WF = \frac{1 - 10^{-a \times l}}{a \times l \times ln(10)} \tag{4.5}$$

where a is the absorbance of the sample for a 1 cm path length at the weighted average (UV LEDs) or peak (LP lamp) wavelength and l is the sample depth. The absorbance was less than 0.02/cm for all experiments, therefore the WF was not weighted to the spectral power distribution for the polychromatic UV LEDs (Kheyrandish *et al.*, 2018).

Divergence factor (DF) Assuming a point source for both the UV LEDs and the LP lamp, the DF was calculated as

$$DF = \frac{D}{D + l} \tag{4.6}$$

where D is the distance from the UV source to the sample surface. To confirm the point source

assumption was valid for the UV LEDs at the desired distance D, the DF was also calculated based on its definition as the ratio of the average irradiance inside the Petri dish in the absence of the sample to the average irradiance at the sample surface. The weighted average irradiance was calculated at $2\,\mathrm{mm}$ intervals from D to $D + l$ and integrated across the depth of the sample and the DF was calculated using the equation determined by Kheyrandish *et al.* (2018)

$$DF_{definition} = \frac{\int_D^{D+l} \overline{E^0}(z)dz}{l \times E^0(D)} \tag{4.7}$$

where $\overline{E^0}$ is the weighted average irradiance measured at depth z. The difference between the calculations was approximately 1% for all UV LEDs, confirming that the point source assumption was valid.

Reflection factor (RF)   The refractive indices of air and water are a function of wavelength, however over the germicidal range and at relatively long distances from the UV source, the impact of wavelength is negligible (Kheyrandish *et al.*, 2018). Therefore the RF was taken to be 0.975 for both UV LEDs and the LP lamp.

Applying the above correction factors to the weighted average incident irradiance and assuming collimation of the beam, the average fluence rate inside the sample for the UV LEDs was calculated as

$$\overline{E_0} = \overline{E^0\left(r, \theta\right)} \times SF \times WF \times DF \times RF \tag{4.8}$$

For the LP lamp the correction factors were applied to the incident irradiance measurement recorded at the centre of the Petri dish and the average fluence rate inside the sample was calculated as

$$\overline{E_0} = E^0(0,0) \times PF \times WF \times DF \times RF \tag{4.9}$$

Exposure times (s) were calculated by multiplying the required fluence $(\mathrm{mJ/cm^2})$ by the inverse of the average fluence rate $(\mathrm{mW/cm^2})$ in the sample. The average fluence rates were $0.053\,\mathrm{mW/cm^2}$, $0.199\,\mathrm{mW/cm^2}$, and $0.692\,\mathrm{mW/cm^2}$ for $255\,\mathrm{nm}$, $265\,\mathrm{nm}$, and $285\,\mathrm{nm}$ UV LEDs respectively and $0.214\,\mathrm{mW/cm^2}$ for the $253.7\,\mathrm{nm}$ LP lamp. Example calculations for both the UV LEDs and the LP lamp can be found in Appendix B.

4.3.4.2   Actinometry

For quality control, radiometry measurements were periodically checked using iodide-iodate $(\mathrm{KI/KIO_3})$ actinometry. The $\mathrm{KI/KIO_3}$ actinometer is useful for measuring the fluence rate from UV-C sources because it does not measurably absorb light above $330\,\mathrm{nm}$ and is therefore optically transparent to ambient lighting. The overall photochemical reaction is

$$8\,\mathrm{I^-} + \mathrm{IO_3}^- + 3\,\mathrm{H_2O} + \mathrm{h}\nu \longrightarrow 3\,\mathrm{I_3}^- + 6\,\mathrm{OH^-}$$
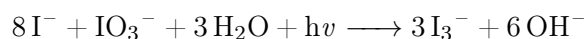
where $\mathrm{I_3^-}$ is a coloured photoproduct which absorbs strongly in UV range and can be accurately quantified at $352\,\mathrm{nm}$. The quantum yield of this actinometer is strongly wavelength dependent and it has therefore primarily been used with LP lamps which are monochromatic (Bolton *et al.*, 2011).

However it can also be used to measure the fluence rate from polychromatic sources, such as the UV LEDs, if the quantum yield is corrected based on the spectral power distribution of the source (Kheyrandish *et al.*, 2018). All available quantum yield data was gathered from the literature and the average values were calculated (Figure 4.5).
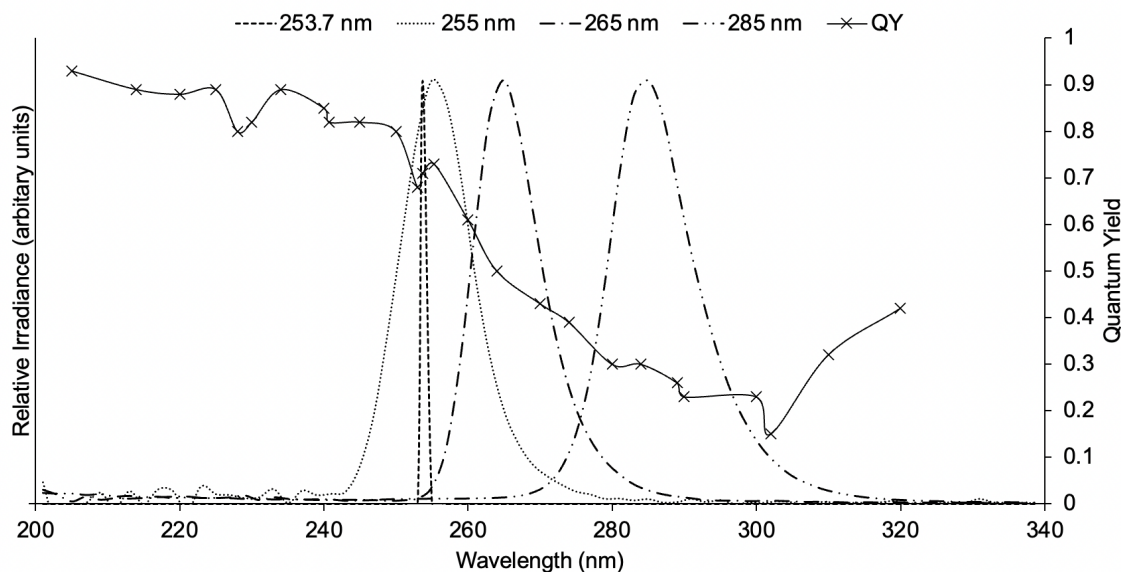
Figure 4.5 Spectral power distribution of the low pressure mercury arc lamp and ultraviolet light-emitting diodes and the quantum yield (QY) of the iodide-iodate actinometer. × denotes average value at given wavelength, calculated from the data available in the literature (Goldstein and Rabani, 2008).

A $0.6\,M\,KI/0.1\,M\,KIO_3$ actinometry solution was prepared and the absorbance of the unexposed solution was measured. A $3\,cm$ diameter Petri dish containing a magnetic stir bar was filled to the brim ($6.6\,mL$) with actinometry solution and exposed to UV light at the required wavelength for 3 minutes. The absorbance of the exposed solution at 352 nm was measured immediately. This procedure was repeated twice more, then the absorbance of the unexposed solution was measured again. The absorbance of the exposed and unexposed solutions was averaged and the difference between them calculated (refer to Appendix B for detailed protocol). The fluence rate was then calculated by integrating over the spectral power distribution of the UV LEDs using the equation below from Kheyrandish *et al.* (2018)

$$\overline{E_0} = \frac{\int \frac{\Delta\alpha_{352} \times v \times U_\lambda \times E_\lambda}{\varepsilon_{352} \times t \times \Phi_\lambda \times A} d\lambda}{\int E_\lambda d\lambda} \qquad (4.10)$$

where $\Delta\alpha_{352}$ is the change in absorbance of the actinometry solution at $352\,nm$ following exposure to UV light, v is the volume of solution (cm^3), U_λ is the photon energy at wavelength λ, E_λ is the spectral irradiance of the UV source at wavelength λ, ε_{352} is the molecular absorption coefficient of the actinometry solution at $352\,nm$ ($27600\,M^{-1}cm^{-1}$), t is the exposure time (s), Φ_λ is the quantum yield at at wavelength λ, and A is the surface area of the Petri dish (cm^2).

The actinometry and radiometry fluence rates were found to be within acceptable limits ($<10\%$ difference (Bolton *et al.*, 2011)) for all sources, with the exception of the 285 nm LEDs for which the percentage difference was 16.8% on average. This higher percentage difference was attributed to inaccuracies in the KI/KIO_3 quantum yield values over 290 nm, which are not well documented, resulting in an underestimation of the 285 nm LED fluence rate when calculated using actinometry. Ferrioxilate actinometry may be better suited to longer wavelength UV LEDs as the quantum yield it is very stable

between 260 nm and 360 nm (Oguma *et al.*, 2016). However because the ferrioxilate system readily absorbs visible light, it must only be used in the dark or under red light (Goldstein and Rabani, 2008), and therefore was not suitable for analysis in RPL.

4.3.5 Ultraviolet exposure procedure

Once the exposure times had been calculated (using radiometry measurements), 3.8 mL suspensions of bottled spring water (0.6 cm deep, 3 cm diameter) containing approximately 100–120 cercariae were exposed at a distance of 10.4 cm from the LED source and 31.5 cm from the LP lamp for the required time. Samples were stirred during the exposure using a magnetic stirrer (Hanna Instruments, UK) and a Teflon® coated stir bar (7 mm long, 2 mm diameter), care was taken to ensure the stirring speed was set so that a vortex did not form on the surface of the suspension. Experiments were carried out at room temperature (18–20 °C). Samples were exposed in a random order to a UV fluence of either 0, 50, 100, 150, 200, or 300 mJ/cm^2 (in duplicate or triplicate), to generate a UV fluence-response curve for each wavelength.

A manual shutter was used to control the exposure times which varied from 1 minute 11 seconds to 95 minutes 19 seconds, depending on the fluence and wavelength of light being used. As the optical power output of the UV LEDs increases with wavelength, the exposure times for the 255 nm LEDs were much longer than for the 285 nm LEDs (e.g. 31 minutes 38 seconds and 2 minutes 27 seconds to achieve a fluence of 100 mJ/cm^2 using the 255 nm and 285 nm LEDs, respectively). The procedure for control samples (0 mJ/cm^2) was the same as for test samples, except the shutter remained closed. Control samples were "exposed" for 10 minutes. All experiments were conducted at least twice, using cercariae shed from different snail batches.

4.3.6 Enumeration of cercariae post exposure

Samples were examined beneath a stereo-microscope after UV exposure and the motility and morphology of cercariae on the bottom of the Petri dish were studied to determine if they were alive or dead. A dissecting needle was used to gently nudge cercariae to see if they would move. Non-moving cercariae that had turned opaque with everted suckers and had fully relaxed tails, often separated from the head, were considered dead. Cercariae that were moving or had contracted, rigid tails with tightly curled forks were considered alive. The number of dead cercariae were counted using a a clicker counter.

In initial experiments samples were only studied immediately following exposure to UV light, however it became clear that UV-damaged cercariae may not die immediately, even when exposed to high fluences. The experiments were therefore repeated, and the number of dead cercariae were counted at 0, 60, and 180 minutes (± 3 minutes) post-exposure. Data from initial experiments were analysed with data at 0 minutes post-exposure from subsequent experiments. All samples were kept in the dark between counting. Following final enumeration of dead cercariae at 180 minutes post-exposure, 20 µL of Lugol's iodine was added to the Petri dish to fix and stain all cercariae and the total was counted.

Cercariae sink if they are not actively swimming, therefore only cercariae that were on the bottom of the Petri dish were counted and where heads and tails had separated only heads were counted to avoid double counting. Surface tension would occasionally trap dead cercariae at the surface of the sample,

although this was rare and when it did occur it would only affect one or two cercariae. Cercariae on the sample surface were not included when counting the dead or total number of cercariae.

4.3.7 Statistical analysis

At least four replicate samples were assayed at each fluence and the total number of cercariae per sample ranged from 96 to 143 (mean 112). The number of alive cercariae was calculated by taking the number of dead from the total number of cercariae in each sample and the log_{10} reduction was calculated as $log_{10}\frac{N_0}{N}$, where N_0 is the mean proportion of alive cercariae in the control ($0\,\mathrm{mJ/cm^2}$) samples and N is the proportion of alive cercariae in the experimental samples at the same time point (i.e. 0, 60, or 180 minutes post-exposure). For the log_{10} reduction of control samples at 60 and 180 minutes post-exposure N_0 is the proportion of alive cercariae in the control samples at 0 minutes post-exposure. In samples where there were no surviving cercariae the minimum log_{10} reduction has been determined by assuming one cercaria had survived.

Fluence-response data in the linear region were fit using least squares regression in GraphPad (Prism version 9.0.2) and the log_{10} fluence-based inactivation rate constant was determined using the equation

$$log_{10}\frac{N_0}{N} = k_D \times F_\lambda + c \qquad (4.11)$$

where k_D is the inactivation rate constant, and F is the fluence at wavelength λ. All data sets exhibited a shoulder at low fluences therefore the fluence-response data were fit with a constant term c, representing the y-intercept. Samples that had no surviving cercariae were excluded from the regression along with data points that were in the shoulder region of the fluence response curve (determined visually).

Inactivation rate constants were compared using a one-way ANOVA ($\alpha = 0.05$) followed by Tukey's multiple comparisons test. If the inactivation rate constants of two wavelengths were statistically similar ($p > 0.05$), an extra-sum-of-squares F-test ($\alpha = 0.05$) was carried out to determine if the two data sets could be fit to a shared model, to confirm similarity.

To assess the impact of stirring at long exposure times a test was also carried out to compare samples kept in the dark for 10, 30, and 90 minutes; half of the samples were stirred and half were unstirred. Dead cercariae were counted at 0, 60, and 180 minutes after stirring, and all samples were kept in the dark (unstirred) between counts. The proportion of alive cercariae in stirred and unstirred samples was compared using an unpaired two-tailed t-test ($\alpha = 0.05$).

4.4 Results

4.4.1 Visible damage caused by ultraviolet exposure

Non-UV exposed cercariae in control samples were observed actively swimming near the sample surface with their tails thrusting quickly from side to side, giving the appearance of spinning (Figure 4.6). When kept in the dark between counting, cercariae became sedate and sunk to the bottom of the Petri dish, however when exposed to bright visible light under the microscope they were quickly stimulated

and swam back to the surface. Only cercariae heads that had separated from the tails would remain on the bottom of the Petri dish, however they were few in number and mostly still alive. It is thought that these heads became separated either during pipetting or because of stirring.

(a)   (b)   (c)

Figure 4.6 Series of photos showing **(a)** tail thrusting, **(b)** contraction, and **(c)** stretching of a *Schistosoma mansoni* cercaria from a control sample ($0\,\text{mJ/cm}^2$), $40\times$ magnification.

Following exposure to increasing fluence of UV light at all wavelengths, cercariae became lethargic and swimming slowed. Periods of non-swimming exceeded periods of swimming, and cercariae began to sink, eventually reaching the bottom of the Petri dish. Here they attempted to swim, but movements were slow or in short burst of twitching, and they were unable to swim back to the surface. Some tails detached but this did not necessarily indicate death and cercariae heads would continue to contract and stretch. Where tails remained attached, they often appeared rigid, with tightly curled forks (Figure 4.7).

At higher fluences, and as time post-exposure increased, movement slowed further and became even more intermittent as the oral and ventral suckers began to invert and evert. Cercariae became sticky, possibly because of gland contents being expelled from the suckers or released at the head-tail junction when detachment occurred. Dead cercariae had opaque, swollen heads with fully everted suckers. This changed the shape of the head, so it appeared slightly triangular at low magnification. The tails became fully relaxed, floating slightly above the head, and easily detached if the sample was disturbed or the cercariae were nudged with a needle. Morphological changes were the same at all wavelengths, but they did not necessarily occur at the same fluence or time post-exposure. Cercariae exposed to $50\,\text{mJ/cm}^2$ did not appear to be immediately damaged at $253.7\,\text{nm}$ or $255\,\text{nm}$, however many alive cercariae had detached tails 60 minutes post-exposure. At $265\,\text{nm}$ and $285\,\text{nm}$ the damage caused by $50\,\text{mJ/cm}^2$ was more immediate, although a small number of cercariae were still actively swimming at 60 minutes post-exposure. The majority of cercariae were visibly damaged immediately following exposure to $100\,\text{mJ/cm}^2$ at all wavelengths, however after 3 hours many were still alive in the $253.7\,\text{nm}$ and $255\,\text{nm}$ samples.

4.4.2   Fluence response of *Schistosoma mansoni* cercariae

The mean fluence-response of *S. mansoni* cercariae to UV exposure at four wavelengths using UV LEDs and a LP lamp at 0, 60, and 180 minutes post-exposure is shown in Figure 4.8, with error bars representing one standard deviation. All data points for 0, 60, and 180 minutes post-exposure are shown in Figures B.1–B.3 in the appendices. The inactivation rate constants and interpolated fluences required to achieve a 1- and 2-$\log_{10}$ reduction at each wavelength and time point are shown in Table 4.2

(a)

(b)

~ 100 µm
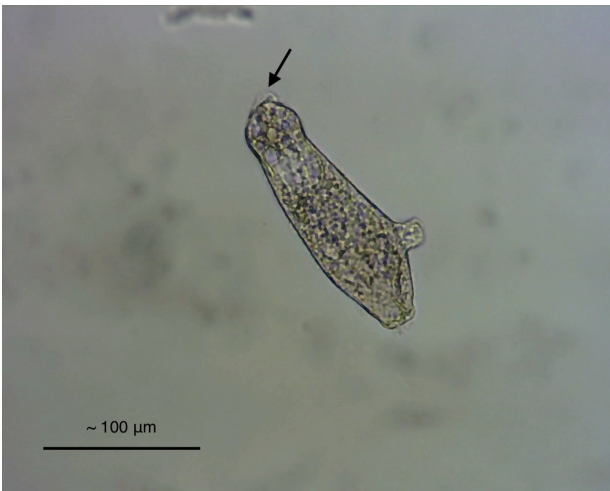
(c)

(d)

Figure 4.7 (a) and (b) Alive but damaged *Schistosoma mansoni* cercaria exposed to $100\,\text{mJ/cm}^2$ at $265\,\text{nm}$ showing rigid tail with tightly curled fork and contraction and stretching of head. **(c) and (d)** Dead *Schistosoma mansoni* cercaria exposed to $300\,\text{mJ/cm}^2$ at $265\,\text{nm}$, showing fully everted oral and ventral sucker with gland contents just visible and fully relaxed, detached tail. All $100\times$ magnification.

(full inactivation equations are shown in the figures in Appendix B).

All wavelengths demonstrated a shoulder at low fluences when studied immediately after exposure (0 minutes). Whilst cercariae were visibly damaged at all wavelengths, the mean maximum $\log_{10}$ reduction achieved immediately following exposure to the highest fluence was 1.6 at 265 nm. In only one sample did all the cercariae die, when exposed to $300\,\mathrm{mJ/cm^2}$ at 265 nm. The inactivation rate constants at 0 minutes post-exposure were statistically different for all UV sources ($p < 0.0001$), except for 255 nm vs 285 nm ($p = 0.45$). A subsequent extra-sum-of-squares F-test confirmed similarity of the curves ($p = 0.23$). Refer to Table B.2 in the appendices for p values for all multiway comparisons.

Table 4.2 Inactivation rate constants (k_D) of *Schistosoma mansoni* cercariae for each wavelength at 0, 60, and 180 minutes post-exposure and the fluence required to achieve 1- and 2-$\log_{10}$ reduction.

			Fluence ($\mathrm{mJ/cm^2}$) (95% CI)	
0 mins	k_D	$\pm$Std. Err	1-$\log_{10}$	2-$\log_{10}$
253.7 nm (LP)	0.0012	0.00017	> 300	> 300
255 nm (LED)	0.0038	0.00039	> 300	> 300
265 nm (LED)	0.0073	0.00050	247 (234-261)	> 300
285 nm (LED)	0.0046	0.00021	> 300	> 300
60 mins	k_D	$\pm$Std. Err	1-$\log_{10}$	2-$\log_{10}$
253.7 nm (LP)	0.0038	0.00033	> 300	> 300
255 nm (LED)	0.0081	0.00048	218 (209-229)	> 300
265 nm (LED)	0.0113	0.00162	127 (111-146)	216 (188-266)
285 nm (LED)	0.0130	0.00141	126 (115-141)	202 (180-239)
180 mins	k_D	$\pm$Std. Err	1-$\log_{10}$	2-$\log_{10}$
253.7 nm (LP)	0.0057	0.00043	208 (194-225)	> 300
255 nm (LED)	0.0105	0.00124	160 (145-179)	255 (226-302)
265 nm (LED)	0.0119	0.00174	99 (85-113)	183 (160-226)
285 nm (LED)	0.0155	0.00163	94 (84-104)	158 (143-181)

Sixty minutes after exposure there was an increase in $\log_{10}$ reduction at all fluences and wavelengths, with the most notable increase at 285 nm. All wavelengths still exhibited a shoulder up to $50\,\mathrm{mJ/cm^2}$. All cercariae were dead in all samples at $300\,\mathrm{mJ/cm^2}$ at 265 nm and 285 nm and in one sample at 255 nm, and in all samples at $200\,\mathrm{mJ/cm^2}$ at 285 nm and in half of the samples at 265 nm. As a result, tailing is observed and the $\log_{10}$ reduction achieved at these fluences should be considered a minimum. The inactivation rate constants at 60 minutes post-exposure were statistically different for all UV sources ($p < 0.05$) except 255 nm vs 265 nm ($p = 0.13$) and 265 nm vs 285 nm ($p = 0.68$). However the F-test comparing 255 nm and 265 nm found the curves should not be fit with the same curve ($p < 0.0001$) and that the y-intercept was statistically different for each wavelength. The similarity of 265 nm and 285 nm was confirmed by the F-test ($p = 0.76$).

$\log_{10}$ reduction continued to increase with time and after 180 minutes there is no longer a shoulder at low fluences at 265 nm and 285 nm, confirming that cercariae were damaged at $50\,\mathrm{mJ/cm^2}$. This suggests that cercariae are not able to repair UV induced damage in the dark, after having been exposed to these fluences. There was also an increase at 253.7 nm and 255 nm at $50\,\mathrm{mJ/cm^2}$, however it was minimal. As with the 60 minutes data, the inactivation rate constants at 180 minutes post exposure were statistically different for all UV sources ($p < 0.05$) except 255 nm vs 265 nm ($p = 0.85$) and 265 nm vs 285 nm ($p = 0.23$). The subsequent F-tests found 255 nm and 265 nm should not be fit with the same curve ($p < 0.0001$) but 265 nm and 285 nm were statistically similar ($p = 0.26$).
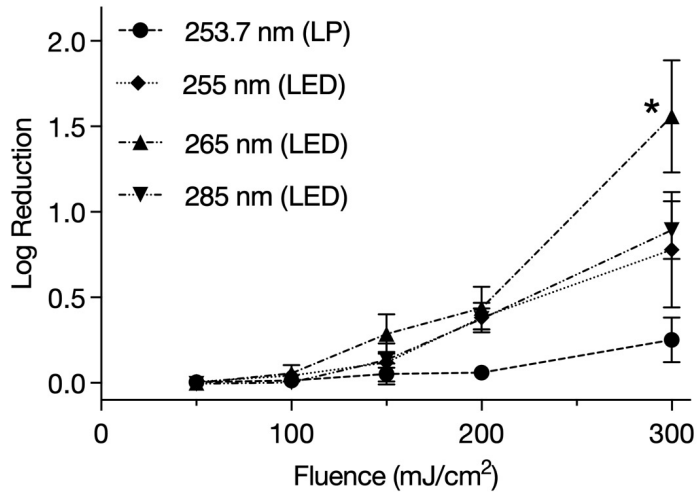
(a) 0 minutes post-exposure
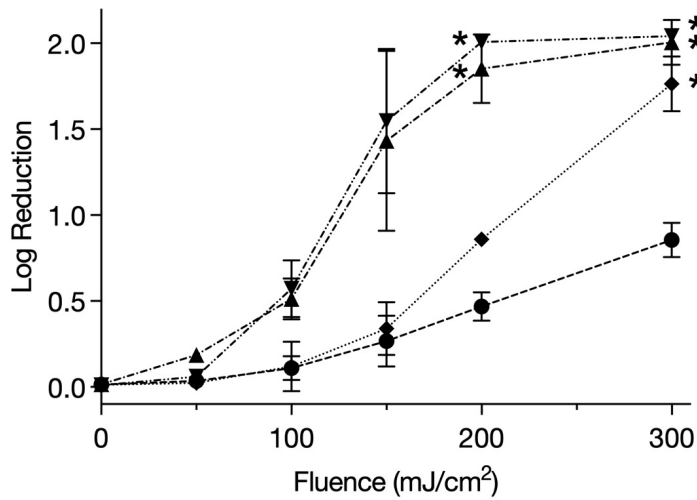
(b) 60 minutes post-exposure
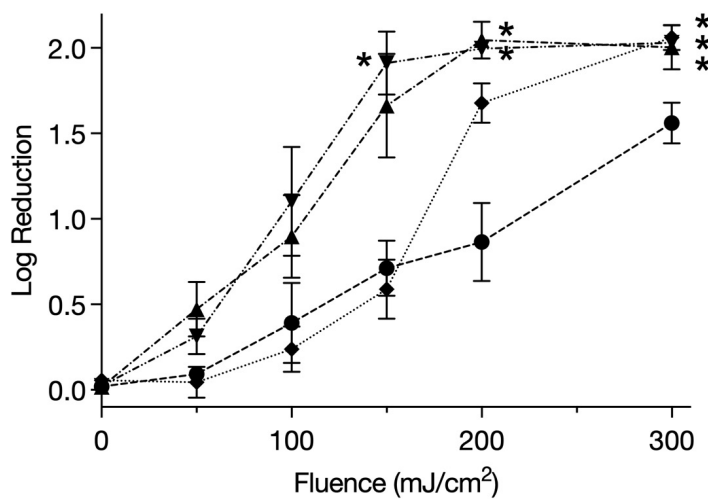
(c) 180 minutes post-exposure

Figure 4.8 Ultraviolet fluence-response of *Schistosoma mansoni* cercariae at **(a)** 0 minutes, **(b)** 60 minutes, and **(c)** 180 minutes after exposure to ultraviolet light at four wavelengths. * indicates all cercariae were dead in at least one sample and therefore represents the minimum $\log_{10}$ reduction achieved. Error bars indicate ± 1 standard deviation.

4.4.3 Effect of stirring

To assess the effect of stirring in the absence of UV light, a test was carried out to compare samples kept in the dark. When studied immediately after stirring, there was a 2% difference between the proportion of live cercariae in samples stirred for 90 minutes when compared to unstirred controls. This increased to 8% and 11% at 60 and 180 minutes after stirring, respectively, however the difference was only statistically significant at 180 minutes when compared with a t-test ($p = 0.007$). The difference between samples stirred for 10 and 30 minutes and unstirred controls was less than 1% and was not significant at any time point ($p > 0.05$).

4.5 Discussion

4.5.1 Ultraviolet sensitivity of *Schistosoma mansoni* cercariae

These results show that very high fluences are required to kill *S. mansoni* cercariae at all the wavelengths studied. When studied immediately after exposure the 265 nm LEDs were the most effective, requiring a fluence of 247 mJ/cm^2 to achieve a 1-log$_{10}$ reduction. This level of inactivation was not achieved at any other wavelength at 0 minutes post exposure; however, the data suggests that cercariae were damaged as there was an increase in log$_{10}$ reduction at 60 and 180 minutes post-exposure at all fluences and wavelengths. This agrees with a previous study by Ariyo and Oyerinde (1990) that found activity of *S. mansoni* cercariae significantly decreased 60 minutes after short exposure to UV light and death rates increased four hours after exposure.

After the 265 nm LEDs, the 285 nm LEDs were most effective, followed by the 255 nm LEDs and finally the LP lamp (253.7 nm). However, there was no statistical difference between the inactivation rate constants at 265 nm and 285 nm after 60 minutes, and slightly higher levels of inactivation were achieved at 285 nm, although all cercariae died in some samples so only the minimum log$_{10}$-reduction achieved at 200 and 300 mJ/cm^2 can be determined for these wavelengths. The fluence response of the LP lamp and 255 nm LEDs is visually similar up to a fluence of 150 mJ/cm^2, but at high fluences 255 nm was more effective and the inactivation rate constants were statistically different, even though the peak wavelengths are very close.

Long periods of stirring (> 30 minutes) in non-UV exposed samples resulted in a statistically significant increase in dead cercariae, suggesting stirring may have contributed to the log$_{10}$ reduction achieved in some UV exposed samples (e.g. samples exposed to 300 mJ/cm^2 at 255 nm). This could explain why the fluence-responses at 253.7 nm and 255 nm diverge at 200 and 300 mJ/cm^2. The average exposure times at 255 nm at these fluences were 62 minutes 50 seconds and 94 minutes 48 seconds respectively, compared to 16 minutes 31 seconds and 24 minutes 47 seconds at 253.7 nm. Cercariae are non-feeding, and they are shed from snail hosts with finite glycogen stores that are used for host finding and skin penetration (Lawson and Wilson, 1980). They are stimulated by water turbulence (Curwen and Wilson, 2003), so it is possible that they utilise their glycogen stores more quickly in stirred samples and die earlier. Alternatively, the difference in efficacy could be due to the polychromatic output of the 255 nm LEDs compared to the monochromatic LP lamp. The emission spectrum of the 255 nm LEDs extends beyond 270 nm (Figure 4.3), with a considerable output at 260–265 nm, the range of

maximum absorption of UV light by the pyrimidine bases.

4.5.2 Ultraviolet disinfection mechanism

The fluences required to achieve a 1- to 2-$\log_{10}$ reduction in cercariae in this study were much higher than required to achieve the same reduction in worm burden in previous vaccine studies examined in Chapter 3 (5–14 mJ/cm^2 at 253.7 nm for a 1-$\log_{10}$ reduction, although fluences should be considered approximate (Lin *et al.*, 2011; Tian *et al.*, 2010; Shi *et al.*, 1990; Kumagai *et al.*, 1992; Dean *et al.*, 1983; Kamiya *et al.*, 1993; Ruppel *et al.*, 1990; Wang *et al.*, 1993; Fang-Li *et al.*, 1996; Hazell *et al.*, 2019)). Similar differences have been observed in other helminths such as *Ascaris suum*, where 100 mJ/cm^2 was required to achieve a 1-$\log_{10}$ reduction in viable embryos but only 11 mJ/cm^2 was required to achieve a 1-$\log_{10}$ reduction in worm burden (Brownell and Nelson, 2006; Tromba, 1978). The difference between these studies was attributed to a delay in the expression of UV-induced DNA damage during cell replication and incomplete recovery of worms from the animal host (Brownell and Nelson, 2006). A SEM study with *S. mansoni* found that worms that developed from cercariae exposed to UV light at 253.7 nm for 2 minutes had abnormal features and damaged teguments (Mohammed, 1999), whilst a SEM study on cercariae exposed to the same wavelength for 2–3 minutes found that irradiated cercariae were morphologically the same as controls (Dajem and Mostafa, 2007). The UV fluences were not recorded in either study, but the authors suggested that the abnormalities in adult worms were a result of either UV-induced DNA damage causing mutagenic effects that do not appear until the adult stage or an enhanced immune response to irradiated cercariae by the host. However, schistosome cercariae do not undergo DNA replication or transcription until they transform into schistosomula upon skin penetration (Roquis *et al.*, 2015), so DNA damage is unlikely to be the cause of visible damage or death of cercariae.

Whilst cercariae do not undergo transcription, RNA is present, and it is assumed that transcripts are formed prior to release from snail hosts, allowing translation to continue (Hagerty and Jolly, 2019). For most organisms (excluding organisms in which RNA is the only genetic material, such as RNA viruses) UV damage to RNA is generally considered inconsequential relative to DNA because it exists in many copies and can be replaced so long as the DNA is unaffected (Harm, 1980). However, in the case of cercariae, RNA cannot be replaced until transformation into schistosomula. A study by Hagerty and Jolly (2019) found that the majority of protein translation occurs in the tails of cercariae, possibly to maintain the energy required for swimming, and treatment with high concentrations of drugs that inhibit protein synthesis reduced activity. RNA damage preventing protein translation could possibly be the cause of visible damage and death of schistosome cercariae and concentration of translation in the tail could possibly explain why head-tail detachment was observed in many samples 60 minutes after exposure to low fluences that did not kill cercariae heads. As RNA exists in many copies within a cell, multiple RNA molecules need to be damaged to prevent cells from functioning, which may explain why such high fluences are required to kill cercariae. Whilst this is speculative, UV-C radiation has been shown to indirectly inhibit translation in mammalian cells (Iordanov *et al.*, 1998; Deng *et al.*, 2002; Wu *et al.*, 2002) and previous studies investigating UV-attenuated cercarial vaccines found that non-lethal UV exposure at 253.7 nm prevented protein synthesis in schistosomula that had been transformed from irradiated cercariae (Wales *et al.*, 1992).

Most bacteria and viruses are less than 2 µm diameter, whereas schistosome cercariae are approximately

400 µm long, 50 µm diameter, and the glycocalyx alone is 1–2 µm thick (Harm, 1980; Caulfield *et al.*, 1988). Therefore, unlike bacteria and viruses, it cannot be assumed that UV light is able to penetrate all parts of the cercaria equally well, and it is possible that it is predominantly absorbed in cells or structures near the surface of the organism (Harm, 1980). UV light has been shown to cause aggregation of the glycocalyx and modify carbohydrates and glycoproteins on the surface of schistosomula transformed from irradiated cercariae (Kusel *et al.*, 1989; Wales and Kusel, 1992; Wales *et al.*, 1993; Modha *et al.*, 1998).

It is likely that exposure to different wavelengths of UV light results in different or multiple disinfection mechanisms. A recent study with *Escherichia coli* found that exposure to UV LEDs with a peak emission at 265 nm caused direct damage to DNA, whereas UV LEDs with peaks at 285 nm and 295 nm caused indirect damage to cellular components through oxidative stress, possibly through the production of intracellular ROS (Pousty *et al.*, 2021). Oxidative stress was also caused at 265 nm and direct DNA damage by the longer wavelengths, but the other mechanisms were dominant. MP mercury arc lamps, which produce polychromatic UV light over a broad spectrum, have also been shown to cause oxidative stress in bacteria (Gerchman *et al.*, 2019) and exposure to filtered sunlight in the UV-A and UV-B range caused both DNA damage and oxidative stress in the cercariae of a marine trematode (Studer *et al.*, 2012). UV exposure can also cause damage to proteins, which strongly absorb UV at wavelengths less than 230 nm with a smaller peak in absorbance at approximately 280 nm (Harm, 1980). Studies have shown that MP lamps and UV LEDs at with peak emissions at 261 nm and 278 nm caused higher levels of protein damage in adenoviruses than LP lamps (Eischeid and Linden, 2011; Beck *et al.*, 2018).

4.5.3 Species

The sensitivity of a microorganism to UV light at different wavelengths is a function of the microorganism itself. Here the effectiveness of UV disinfection against *S. mansoni* cercariae has been examined, which is one of the most widespread human schistosome species, present in South America, the Caribbean, the Middle East, and Africa. However further study of the other main human species, *S. haematobium* and *S. japonicum*, is required to determine if there is a difference in fluence-response. Previous studies did not find any difference in the UV sensitivity of *S. mansoni* and *S. japonicum* cercariae (Ruppel *et al.*, 1990); *S. haematobium* has been shown to be slightly more resistant than *S. mansoni* (Ghandour and Webbe, 1975), although no statistical analysis was performed. Whilst cercariae of the human schistosomes are physiologically similar, differences in behaviour and genomic differences could result in varying UV sensitivity between species, particularly if damage to nucleic acids is a principal disinfection mechanism.

4.5.4 Measure of inactivation

The maximum $\log_{10}$ reduction achieved in this study was 2.1 representing $> 99\%$ removal. As all the cercariae were dead in these samples it is possible a higher level of inactivation could be reached. This was only achieved 1 to 3 hours post exposure for the UV LEDs, and not at all for the LP lamp. In this study the morphology and motility of cercariae were assessed to determine if they were alive or dead, this is a common method used to assess schistosome cercariae, but it can be subjective. Furthermore, assuming that all living cercariae are still infective even when they are visibly damaged is likely

conservative. It was not possible to confirm if cercariae that died at 60 or 180 minutes post-exposure were infective or viable at 0 minutes post-exposure, but it has been demonstrated previously that older, non-swimming cercariae can still penetrate skin (Lawson and Wilson, 1980), so a conservative approach is considered appropriate from a public health protection standpoint. Future studies could use human skin samples obtained from cosmetic surgery to study skin penetration (Bartlett *et al.*, 2000). Alternatively fluorescence assays could also be used to assess the integrity of cell membranes following UV exposure or immunological assays could be used to detect nucleic acid damage (Braun *et al.*, 2020a; Blyth *et al.*, 2021). These assays have the additional benefit of being objective and they could also provide an insight into the possible UV disinfection mechanisms, they are therefore explored in more detail in the following chapter.

4.5.5 Water matrix

Experiments were carried out under controlled conditions using bottled spring water. Whilst the use of the water factor in the fluence calculation ensures that the fluence-response is independent of the absorbance of the water sample, in turbid water suspended particles may enmesh and physically shield pathogens from UV light (Parker and Darby, 1995; Mamane and Linden, 2006; Winward *et al.*, 2008; Templeton *et al.*, 2008). A previous study found that there was a reduction in the effectiveness of UV disinfection against schistosome miracidia when using 1% turbid water, however as the fluence rate was not measured in this study and the authors did not account for the absorbance of the water samples, it is not possible to separate the effects of absorbance or particle shielding (Prah and James, 1977). Cercariae are relatively large and, unlike miracidia which exhibit positive geoxtaxis and tend to accumulate at the bottom of containers, they are phototactic and tend to be attracted to the water surface (Short and Gagné, 1975). They are therefore less likely to be effected by particle shielding than much smaller pathogens such as bacteria and viruses. Nevertheless it is recommended that experiments are repeated in the field using water collected from local water bodies. If water is of particularly poor quality, pre-treatment (e.g. filtration or sedimentation) may be required to reduce the turbidity of the water.

4.5.6 Cercarial concentration

The concentration of cercariae in water bodies varies significantly and is dependent on many complex factors (2.2.2), but previous studies have reported ranges from less than 0.1 to over 100 cercariae/L (Rowan, 1958; Kloos *et al.*, 1982; Prentice and Ouma, 1984; Ouma *et al.*, 1989; Muhoho *et al.*, 1997; Aoki *et al.*, 2003). This is much lower than the average concentration of cercariae used in this study (29 cercariae/mL) and pre-treatment is likely to reduce the cercarial concentration even further. Therefore the fluence required to achieve a 2-$\log_{10}$ reduction may actually be sufficient to inactivate all cercariae in water that is used for contact activities (e.g. laundry, bathing).

4.6 Conclusions

This is the first reproducible study to examine the direct effect of germicidal UV light on *S. mansoni* cercariae. Very high fluences are required to immediately kill *S. mansoni* cercariae even at the most effective wavelength, requiring $247\,\mathrm{mJ/cm^2}$ (95% CI: 234–$261\,\mathrm{mJ/cm^2}$) to achieve a 1-$\log_{10}$ reduction

at 265 nm. Cercariae were visibly damaged at lower fluences and the fluence required for a 1-$\log_{10}$ reduction reduces to 127 mJ/cm^2 (95% CI: 111–146 mJ/cm^2) and 99 mJ/cm^2 (95% CI: 85–113 mJ/cm^2) if cercariae are stored for 1 and 3 hours after exposure, respectively. At 0 minutes post exposure 285 nm was found to be slightly less effective that 265 nm, but there was no statistical difference after 60 minutes. The least effective wavelengths were 255 nm and 253.7 nm. The fluences required to achieve a 1-$\log_{10}$ reduction in cercariae were considerably higher than required to achieve the same reduction in worm burden in previous vaccine studies (Chapter 3). This is possibly because schistosome cercariae do not undergo any DNA replication or transcription until they transform into schistosomula, therefore any DNA damage induced at lower fluences will not become evident until after skin penetration. Further research is required to investigate the possible UV disinfection mechanisms that are responsible for inactivating and killing these complex microorganisms.

Whilst the concentration of cercariae used in this study is unlikely to occur in the environment, and total death of cercariae may be considered a conservative measure of UV effectiveness, this is the correct approach from a public health standpoint, unless it can be demonstrated that schistosome cercariae that are damaged but not immediately killed by lower UV fluences are completely non-infective or non-viable.

5 Immunological detection of ultraviolet induced DNA damage in *S. mansoni* cercariae

5.1 Abstract

The biological effectiveness of UV disinfection is primarily attributed to photochemical reactions in DNA and RNA which prevent cell replication and therefore render single celled organisms harmless. Schistosomes have much more complex life cycles than bacteria and viruses, and as a result, the UV disinfection mechanism is less clear. Nucleic acid damage in schistosome cercariae was explicitly investigated for the first time using an enzyme-linked immunosorbant assay (ELISA) to detect cyclobutane pyrimidine dimer formation in *S. mansoni* DNA following exposure to UV light. Samples containing 100–300 cercariae were exposed to a range of fluences (0–$150\,\mathrm{mJ/cm^2}$) at three peak wavelengths: $255\,\mathrm{nm}$, $265\,\mathrm{nm}$, and $285\,\mathrm{nm}$ (UV LEDs). Following exposure the cercarial DNA was extracted and the concentration was quantified. An ELISA kit was used to quantified the cyclobutane pyrimidine dimer formation at each fluence and wavelength. Dimers were formed when cercariae were exposed to $50\,\mathrm{mJ/cm^2}$ at all wavelengths studied, and at even lower fluences at the most effective wavelengths ($255\,\mathrm{nm}$ and $265\,\mathrm{nm}$). The results suggest that *S. mansoni* cercariae are possibly made non-viable at much lower fluences than is required to kill them, as dimers in the parasite DNA would prevent cell replication and development inside the host. However, cercariae may still be able to penetrate the skin, and it was not within the scope of the study to confirm how many dimers (or what fluence) is required to ensure the death of cercariae following penetration. Further research is required to investigate if the fluences presented here are sufficient for making water safe for contact activities by confirming if cercariae are either non-infective or non-viable.

5.2 Introduction

The UV disinfection mechanism of single celled microorganisms and viruses that are exposed to monochromatic light from a LP lamp has been extensively studied and is overall very well understood. In short, dimerization of the nucleic acids prevents cell replication, thereby rendering the pathogen inactivated. With the introduction of polychromatic MP lamps and UV LEDs which emit across the germicidal range, studies have investigated other UV disinfection mechanisms such as protein damage and oxidation by intracellular ROS, however these have also primarily focused on bacteria and viruses (Ou *et al.*, 2011; Eischeid and Linden, 2011; Li *et al.*, 2017; Beck *et al.*, 2018; Gerchman *et al.*, 2019; Pousty *et al.*, 2021). In contrast, there has been little research into the UV disinfection mechanisms of multicellular organisms such as helminth eggs and larvae, which have far more complex life cycles and are much larger organisms compared to single celled pathogens. Studies on singled celled *Ascaris* spp. eggs have demonstrated that UV exposure at $253.7\,\mathrm{nm}$ prevents cell replication and embryonation (Lucio-Forster *et al.*, 2005; Brownell and Nelson, 2006), suggesting a similar mechanism to the inactivation of bacteria and viruses. However, unlike ascaris eggs, schistosome cercariae do not undergo DNA replication or transcription until they have transformed into schistosomula (Roquis *et al.*, 2015).

Some immunisation studies have shown that exposing schistosome cercariae to UV light at sub-lethal fluences causes morphological mutations in adult worms, but this could be a result of DNA damage or an enhanced immune response in the host (Mohammed, 1999). To date nucleic acid damage in schistosome cercariae has not been explicitly demonstrated or investigated in detail.

The experiments presented in the previous chapter demonstrated that the UV fluence-response of *S. mansoni* cercariae was dependent on wavelength, however there was no statistical difference between cercariae exposed to 265 nm and 285 nm when they were studied one hour after exposure. This is surprising given that 265 nm is much closer to the peak absorbance of nucleic acids than 285 nm and suggests that multiple disinfection mechanisms may play a role in the UV inactivation of schistosome cercariae when they are exposed to polychromatic light. A study on the effects of UV-A and UV-B light on *Maritrema novaezealandensis* cercariae, a marine trematode that has two intermediate hosts (snails and crustaceans) and an avian definitive host, found that UV exposure induced DNA dimerization and caused oxidative damage to proteins (Studer *et al.*, 2012). In this chapter the wavelength dependence of the UV disinfection mechanism of *S. mansoni* cercariae is investigated for the first time by examining nucleic acid damage across the germicidal range.

There are a number of methods for detecting UV induced nucleic acid damage and/or dimer formation, including: endonuclease degradation, polymerase chain reaction (PCR), high-pressure liquid chromatography, enzyme-linked immunosorbant assays (ELISA), and bacterial bioreporters (Sutherland and Shih, 1983; Süß *et al.*, 2009; Eischeid *et al.*, 2009; Studer *et al.*, 2012; Sun *et al.*, 2018; Lo *et al.*, 2021; Pousty *et al.*, 2021; Blyth *et al.*, 2021). An ELISA was chosen in this instance because it is a relatively simple technique based on well understood technology and, unlike PCR based detection of dimers, the ELISA provides a positive response to increasing UV fluence so it is well suited to high fluence applications (Blyth *et al.*, 2021). Furthermore, it is a relatively quick, high-throughput assay that is inexpensive and, with exception of a plate reader, only basic lab equipment is required.

The ELISA is an immunological assay that uses antibodies to detect and quantify a specific antigen or other target molecule in biological samples (Crowther, 2000). The antibody is conjugated to an enzyme, or it is bound to a secondary antibody that is conjugated to an enzyme, which reacts with a substrate to produce a visible signal that can be measured, such as a colour change (Figure 5.1). Samples containing unknown quantities of the target antigen are incubated (normally overnight) in a high-binding multiwell plate. Once bound, excess liquid is removed and the wells are washed to remove any unbound material. Unoccupied binding sites around the target are blocked using a buffer solution to prevent non-specific binding of the antibodies to the well. After another wash step the samples are incubated with the primary antibody that is specific to the target antigen. The wells are washed again to remove any unbound primary antibody, then the samples are incubated with the secondary antibody enzyme conjugate. Following a final wash step the substrate solution is added. The antibody bound enzyme catalyses a chromogenic reaction, converting the substrate into a coloured product. The amount of antigen in the sample can then be quantified by measuring the absorbance of each well at a wavelength specific to the substrate.

Immunoassays (ELISA and immuno-blotting) using antibodies that are specific to thymine dimers (anti-thymine antibody), cyclobutane pyrimidine dimers (anti-CPD antibody) and (6-4)photoproducts (anti-6-4PP antibody) have been used to detect dimer formation in DNA from UV-treated water samples from a variety of sources as well as in *M. novaezealandensis* cercariae exposed to UV-A and
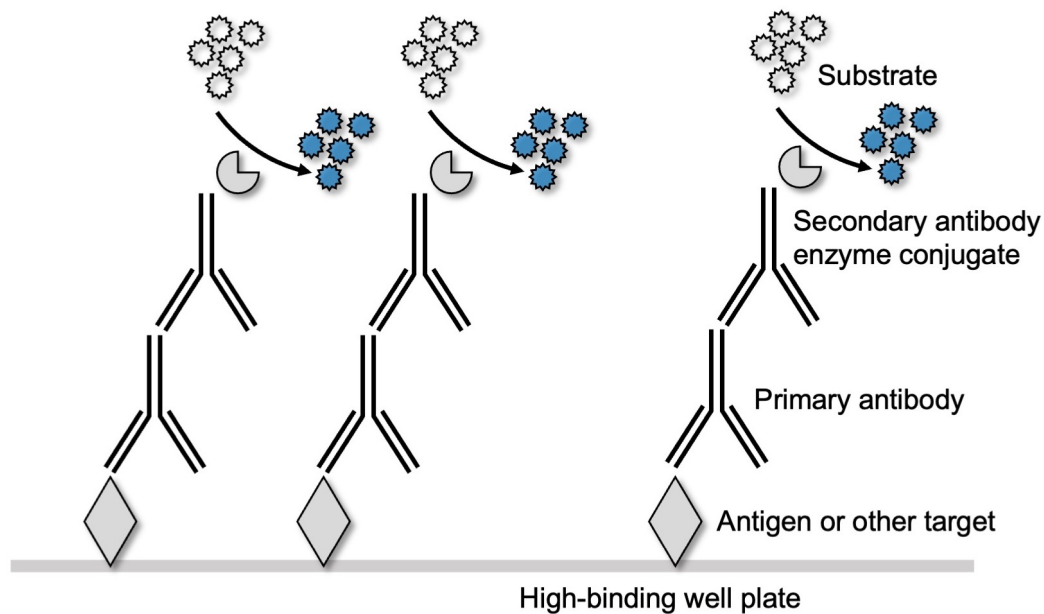
Figure 5.1 Diagram showing the difference components of an enzyme-linked immunosorbant assay. Image produced in Microsoft® PowerPoint for Mac version 16.56.

UV-B light (Roza *et al.*, 1988; Mori *et al.*, 1991; Kraft *et al.*, 2011; Studer *et al.*, 2012; Blyth *et al.*, 2021). There are number of ELISA kits available that are specific for UV dimers in DNA, but because UV-induced RNA damage is generally of less biological significance than DNA damage (Harm, 1980), there are no commercially available antibodies that are specific to uracil or cytosine dimers in RNA. However, as the UV absorption characteristics of RNA resemble those of DNA it can be assumed that dimer formation will also be similar (Harm, 1980; Kowalski *et al.*, 2009). Here the monocolonal anti-CPD antibody that was first raised by Mori *et al.* (1991) is used to detect cyclobutane pyrimidine dimer formation in *S. mansoni* DNA following exposure to polychromatic germicidal UV light at three peak wavelengths: 255 nm, 265 nm, and 285 nm.

5.3 Methods

5.3.1 Ultraviolet exposure procedure

Snail maintenance, preparation and enumeration of cercariae, and fluence determination all proceeded as previously described in Section 4.3. To increase the concentration of DNA the number of cercariae per sample was maximised, depending on the total number that shed each day and the number of exposures to be carried out. Stirred suspensions of 3.8 mL (0.6 cm deep, 3 cm diameter) containing approximately 100–300 cercariae were exposed at distance of 10.4 cm from the LED source for the required time. Samples were exposed in a random order to a UV fluence of either 0, 10, 25, 50, 100, or 150 mJ/cm^2 in duplicate, at 265 nm and 285 nm. Due to the long exposure exposure times required for samples exposed to the 255 nm LEDs, these samples were exposed to only 0, 25, 50, or 100 mJ/cm^2 so that there was sufficient time to carry out the DNA extraction on the same day. Positive control samples were exposed to 500 mJ/cm^2 at 265 nm and 285 nm. A positive control sample at 255 nm was not feasible as the exposure time would have been over 2^1/$_2$ hours. A manual shutter was used to

control the exposure times which varied from 13 seconds to 39 minutes 57 seconds, depending on the fluence and wavelength of light being tested. The procedure for control samples ($0\,\mathrm{mJ/cm^2}$) was the same as for test samples, except the shutter remained closed. Control samples were "exposed" for 10 minutes.

5.3.2 DNA extraction

Once all the UV exposures were complete, samples were transferred to the dark at $4\,^\circ\mathrm{C}$ for 20–30 minutes, causing the cercariae to sink to the bottom of the dish and become immobile. Taking care to collect as many cercariae as possible, $2\,\mathrm{mL}$ was taken from the bottom of each Petri dish and transferred to a clean $2\,\mathrm{mL}$ collection tube via pipette. Collection tubes were centrifuged at 13000 rpm in a microcentrifuge for 2 minutes. The supernatant was removed via pipette, leaving the cercariae in $80\,\mathrm{\mu L}$ of liquid in the bottom of the tube.

DNA extraction proceeded according to the methodology supplied with the DNeasy Blood & Tissue kit (Qiagen, Netherlands), with some minor adaptations that were suggested by Dr Fiona Allan who has previously used this kit to isolate schistosome cercariae DNA. Samples were first lysed by adding $90\,\mathrm{\mu L}$ of tissue lysis buffer solution (ALT buffer) and $10\,\mathrm{\mu L}$ of protinase K to each collection tube and vortexing for 10 seconds, before incubating at $56\,^\circ\mathrm{C}$ for 1 hour. Samples were removed from the incubator and vortexed for 15 seconds before adding $100\,\mathrm{\mu L}$ of lysis buffer (AL buffer) and $100\,\mathrm{\mu L}$ of molecular grade ethanol to each tube, vortexing between each addition. The mixture from each tube was pipetted into a DNeasy spin column and centrifuged at 8000 rpm for 1 minute, this binds the DNA to the spin column membrane, whilst allowing all other material to pass through. Two wash steps followed, in accordance with the manufacturer's methodology. The spin columns were transferred to clean $2\,\mathrm{mL}$ collection tubes and $60\,\mathrm{\mu L}$ of elution buffer (AE buffer, warmed to $70\,^\circ\mathrm{C}$) was added. Columns were left to stand for 1 minute at room temperature before centrifuging at 8000 rpm for 1 minute. An additional $60\,\mathrm{\mu L}$ of elution buffer was added to the column and the final spin step was repeated to increase the total yield of DNA.

The DNA concentration was quantified using a Qubit dsDNA high sensitivity assay kit (Thermo Fisher Scientific, USA) which can accurately quantify double strand DNA concentrations from $1\,\mathrm{ng/mL}$ to $500\,\mathrm{ng/mL}$. In accordance with the methodology supplied with the assay kit, a working solution was prepared by diluting the high sensitivity reagent 1:200 in the buffer solution provided. For the DNA standards, $190\,\mathrm{\mu L}$ of the working solution was added to two $500\,\mathrm{\mu L}$ thin walled PCR tubes, followed by $10\,\mathrm{\mu L}$ of the DNA standards. For samples, $198\,\mathrm{\mu L}$ of the working solution was added to as many tubes as required, followed by $2\,\mathrm{\mu L}$ of each sample. Solutions were mixed by vortexing for 2–3 seconds and then incubated at room temperature for 2 minutes. Using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, USA), the two DNA standards were read first to determine the calibration curve, followed by each of the samples. DNA concentrations varied from $8.84\,\mathrm{ng/mL}$ to $19.8\,\mathrm{ng/mL}$ (mean $13.77\,\mathrm{ng/mL}$). All DNA samples were stored at $-20\,^\circ\mathrm{C}$ until required for the ELISA.

5.3.3 Enzyme-linked immunosorbant assay procedure

Cyclobutane pyrimidine dimer formation was quantified using the OxiSelect UV-Induced DNA Damage (CPD Quantitation) ELISA kit (Cell Biolabs, USA). CPD-DNA supplied with the kit was used as

a standard positive control. The concentration of DNA in the test samples was much lower than the recommended concentration of 4 µg/mL, the standard was therefore further diluted to a total DNA concentration of 20 ng/mL with varying concentration of CPD-DNA (0.156–10 ng/mL, diluted in Reduce DNA also supplied with the kit), to confirm that low concentrations of dimerized DNA could be detected by the assay.

DNA samples and standards were first converted to single strand DNA by heating to 95 °C for 10 minutes before rapidly cooling on ice for a further 10 minutes. 50 µL of a sample or standard was loaded in to each well of a 96-well DNA high-binding plate, followed by 50 µL of DNA binding solution. Wells were mixed by pipetting then incubated at room temperature (20 °C) for 16 hours on an orbital shaker at 100 rmp. Each DNA sample and standard were assayed in duplicate. The following morning the DNA solutions were removed and the wells washed twice with phosphate buffered saline solution. Following each wash step the plate was blotted on paper towels to remove any excess liquid. 200 µL of assay diluent was added to each well and the plate was incubated without shaking at room temperature for one hour. The assay diluent was removed and the plate blotted on paper towels. The primary anti-CPD antibody was diluted 1:1000 in the assay diluent and 100 µL of the diluted antibody solution was added to each well. The plate was then incubated for one hour at room temperature on an orbital shaker (100 rpm). All wells were washed five times using 250 µL of the wash buffer provided with the assay kit, with thorough blotting after each wash to remove excess liquid. The secondary antibody-HRP conjugate was diluted 1:1000 in the assay diluent and 100 µL was added to each well before incubating again for one hour at room temperature on the orbital shaker. Following this final incubation step the wells were washed a further five times with the wash buffer. The substrate solution was warmed to room temperature and 100 µL was added to each well. In the presence of dimerized DNA a blue coloured product is produced. The absorbance was read every two minutes for 28 minutes using a FluorStar OMEGA (BGM Labtech) plate reader at 620 nm. Mixing was by orbital shaking at 500 rpm for 1 minute before the first absorbance measurement.

5.3.4   Statistical analysis

The absorbance (or optical density, OD) data were normalised by dividing by the the DNA concentration for each sample. Both endpoint and initial reaction rates were used to analyse dimer formation. The endpoint of the reaction was determined by plotting the OD data over time. The time at which the first sample saturated (i.e. the end of the linear region of the kinetic data) was taken as the endpoint. For the initial rate analysis the kinetic data for each sample were fit with a 2nd order polynomial using least squares regression and the rate of change of OD_{620} at 0 minutes was evaluated. The data were plotted over fluence and fit using least squares regression in GraphPad (Prism version 9.0.2). The fluence-based rate constant was determined using the equation

$$y = k \times F_\lambda + c \tag{5.1}$$

where y is either the endpoint OD_{620} per unit DNA or the rate of change of OD_{620} per unit DNA at 0 minutes, k is the fluence-based reaction rate constant, and and F is the fluence at wavelength λ. All data sets exhibited a background response at $0 \, mJ/cm^2$ therefore the OD data was fit with a constant term c, representing the y-intercept. Rate constants at 255 nm, 265 nm, and 285 nm were compared using a one-way ANOVA ($\alpha = 0.05$) followed by Tukey's multiple comparisons test. If the inactivation

rate constants of two wavelengths were statistically similar ($p > 0.05$), an extra-sum-of-squares F-test ($\alpha = 0.05$) was carried out to determine if the two data sets could be fit to a shared model, to confirm similarity.

5.4   Results

Fluence-dependent dimer formation was detected in samples exposed to all three wavelengths tested. Positive control samples exposed to $500\,\text{mJ/cm}^2$ at $265\,\text{nm}$ and $285\,\text{nm}$ displayed the strongest response, as expected, however even at relatively low fluences (10–$25\,\text{mJ/cm}^2$) there was a just visible colour change in samples exposed to the $255\,\text{nm}$ and $265\,\text{nm}$ LEDs (Figure 5.2).
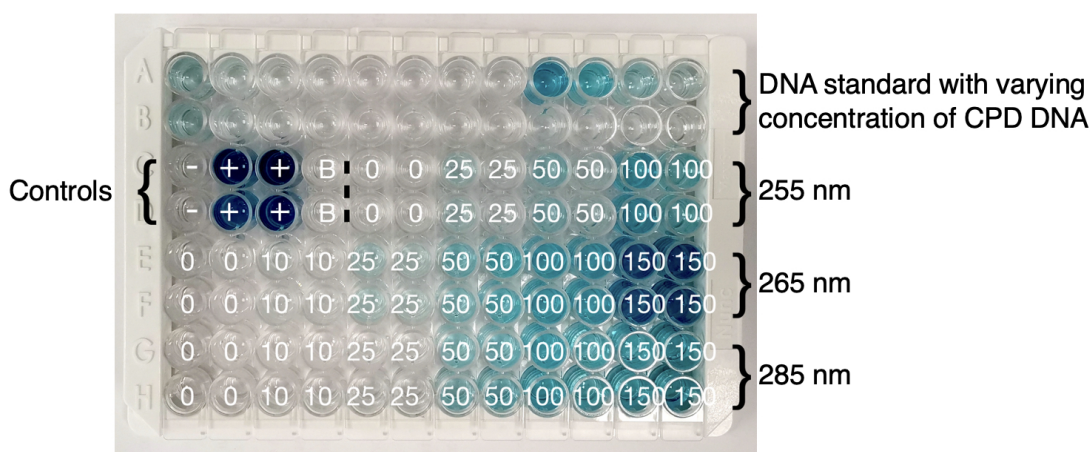
Figure 5.2 Photograph of the enzyme-linked immunosorbant assay plate taken immediately after reading. "–" denotes negative control, "+" denotes positive control (exposed to $500\,\text{mJ/cm}^2$ at $265\,\text{nm}$ (wells C2 and D2) and $285\,\text{nm}$ (wells C3 and D3)), "B" denotes blank, and numbers indicate the fluence each sample was exposed to. There is a strong colour change in the positive control samples and in the test samples exposed to $150\,\text{mJ/cm}^2$ at $265\,\text{nm}$ (wells E11, E12, F11, and F12).

Both initial reaction rate and endpoint data were used to analyse dimer formation at each wavelength. There were some inconsistencies in the initial measurements which lead to OD_{620} values being higher at 0 minutes than at 2 minutes in some samples. This resulted in very high coefficients of variation (CV) in the initial reaction rate data for some of the fluences (Table 5.1), and as a result the coefficients of determination for the linear regression were low ($\text{R}^2 = 0.31$, 0.68, 0.51 for $255\,\text{nm}$, $265\,\text{nm}$, and $285\,\text{nm}$, respectively). Therefore only the endpoint data was analysed further. The initial rate data are shown in Figure 5.3.

The endpoint was taken as the time point at which the first sample saturated and was found to be 14 minutes. The mean OD_{620} per unit DNA at each wavelength and fluence, and associated CV values, are shown in Table 5.2. The CV values are relatively high in some samples however it was still possible to detect a linear response at all wavelengths with a reasonable goodness of fit ($\text{R}^2 = 0.72$, 0.81, 0.70 for $255\,\text{nm}$, $265\,\text{nm}$, and $285\,\text{nm}$, respectively). The ELISA endpoint data demonstrating fluence- and wavelength-dependent dimer formation in UV exposed *S. mansoni* DNA is shown in Figure 5.4.

Samples exposed to the $265\,\text{nm}$ LEDs exhibited the strongest response, however there was no statistical difference between the $255\,\text{nm}$ and $265\,\text{nm}$ endpoint rate constants ($p = 0.28$) and a subsequent extra-sum-of-squares F-test confirmed similarity of the curves ($p = 0.48$). The $285\,\text{nm}$ rate constant

was significantly different to both the 255 nm and 265 nm rate constants ($p = 0.03$ and $p < 0.0001$, respectively), suggesting this wavelength is least effective at inducing cyclobutane pyrimidine dimer formation.

Table 5.1 Mean initial rate of change of absorbance per unit DNA and associated coefficient of variation.

Fluence (mJ/cm^2)	255 nm		265 nm		285 nm	
	d/dt OD_{620}	CV (%)	d/dt OD_{620}	CV (%)	d/dt OD_{620}	CV (%)
0	0.0006	38	0.0004	71	0.0002	268
10	-	-	0.0004	278	-0.0001	-292
25	0.0005	82	0.0004	40	0.0002	113
50	0.0006	64	0.0008	90	0.0009	44
100	0.0013	53	0.0014	39	0.0011	39
150	-	-	0.0030	22	0.0011	18

Table 5.2 Mean endpoint absorbance per unit DNA at $t = 14$ minutes and associated coefficient of variation.

Fluence (mJ/cm^2)	255 nm		265 nm		285 nm	
	OD_{620}	CV (%)	OD_{620}	CV (%)	OD_{620}	CV (%)
0	0.008	25	0.007	35	0.009	73
10	-	-	0.011	53	0.007	41
25	0.012	33	0.006	17	0.006	16
50	0.013	21	0.019	37	0.011	12
100	0.030	27	0.022	31	0.022	18
150	-	-	0.054	9	0.021	13
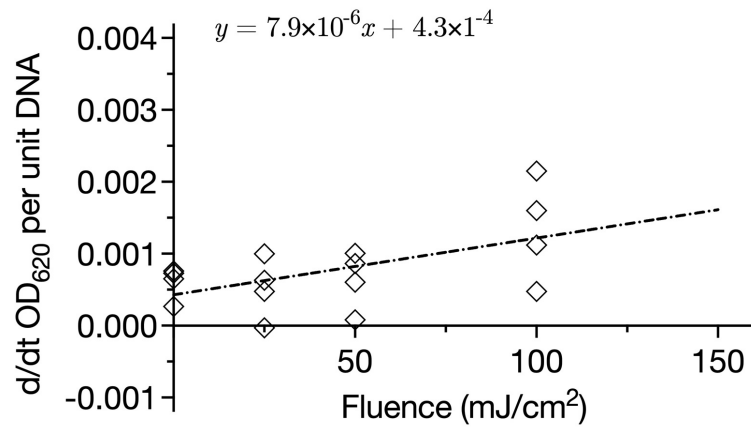
(a) 255 nm
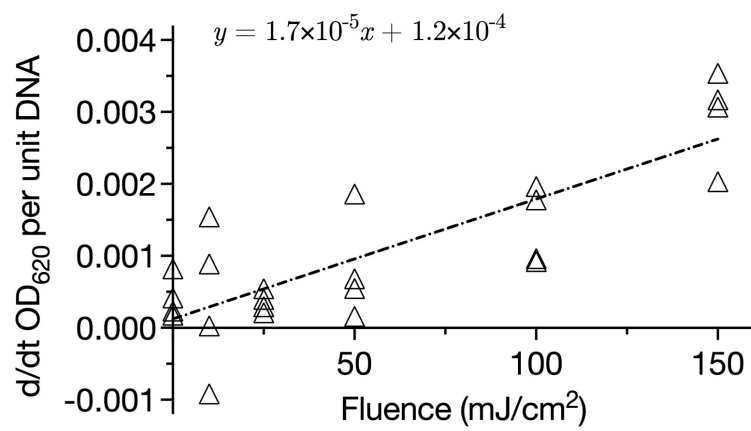
(b) 265 nm
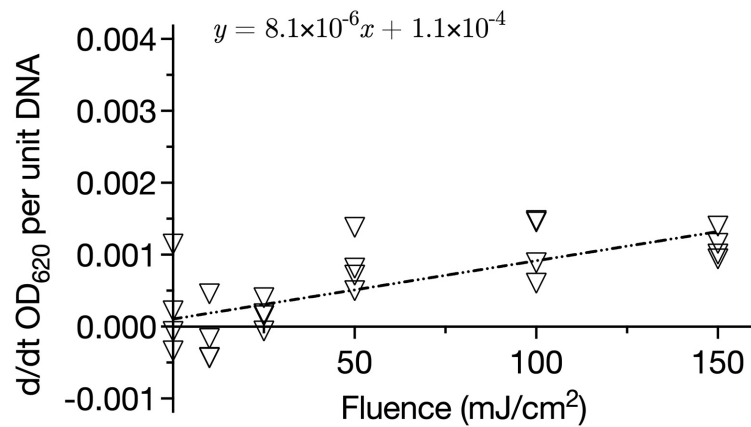
(c) 285 nm

Figure 5.3 Fluence-response of *S. mansoni* DNA after cercariae were exposed to UV LEDs with peak wavelengths at **(a)** 255 nm, **(b)** 265 nm, and **(c)** 285 nm – initial rate data. Equation represents initial rate of change of OD_{620} per unit DNA at $t = 0$ minutes (y) as a function of fluence (x).
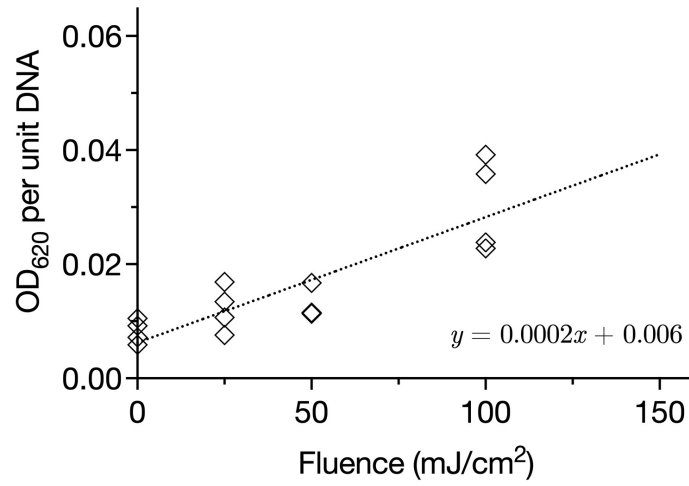
(a) 255 nm
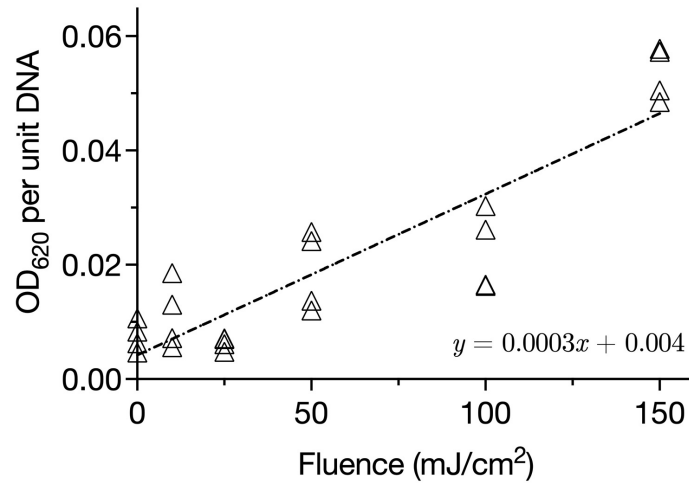
(b) 265 nm
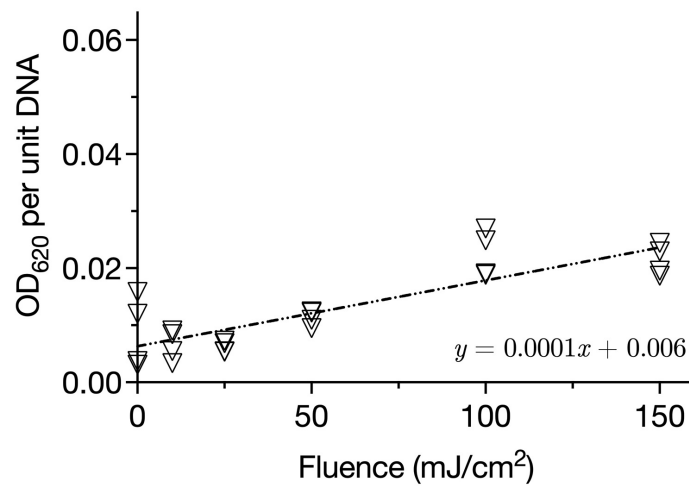
(c) 285 nm

Figure 5.4 Fluence-response of *Schistosoma mansoni* DNA after cercariae were exposed to ultraviolet light at peak wavelengths of **(a)** 255 nm, **(b)** 265 nm, and **(c)** 285 nm – endpoint data. Equation represents endpoint OD_{620} per unit DNA at $t = 14$ minutes (y) as a function of fluence (x).

5.5 Discussion

5.5.1 Ultraviolet induced dimer formation in *Schistosoma mansoni* DNA

The ELISA results explicitly demonstrate for the first time that cyclobutane pyrimidine dimers are formed in *S. mansoni* DNA following UV exposure. This confirms that even though schistosome cercariae are relatively large microorganisms, UV light does penetrate the surface structures and is absorbed by critical components within the cells. Whilst there were some limitations, this is the first time an ELISA has been used to establish a fluence-response in helminth DNA exposed to germicidal UV light, demonstrating the applicability of this technique for studying the effectiveness of UV disinfection in non-culturable organisms.

Cyclobutane pyrimidine dimers were formed when cercariae were exposed to a fluence of $50\,\text{mJ/cm}^2$ at all wavelengths tested, and at $255\,\text{nm}$ and $265\,\text{nm}$ they were formed at lower fluences in some samples. This confirms that, even though cercariae may not be visibly damaged, exposure to UV light at low fluences does induce damage on a molecular level. Furthermore, whilst the fluence-response study in Chapter 4 demonstrated that fluences in excess of $247\,\text{mJ/cm}^2$ are required to immediately kill *S. mansoni* cercariae, the data presented here suggests they may be made non-viable at lower fluences. This is because when cercariae penetrate the skin and transform into schistosomula DNA transcription is rapidly resumed in the first 3–4 hours after penetration, and cell replication begins within 18 hours (Roquis *et al.*, 2015). These processes will stall if a sufficient number of cyclobutane pyrimidine dimers are formed, resulting in the death of some cells and possibly the death of the parasite. This may explain why a previous study reported significant increases in mortality during skin penetration after short exposures to UV light that did not visible damage cercariae (Ghandour and Webbe, 1975) and why in the immunisation studies reviewed in Chapter 3 there were significant reductions in worm burden following exposure to fluences much lower than those required to kill cercariae in the experiments presented in Chapter 4 (Dean *et al.*, 1983; Shi *et al.*, 1990; Ruppel *et al.*, 1990; Kumagai *et al.*, 1992; Kamiya *et al.*, 1993; Wang *et al.*, 1993; Fang-Li *et al.*, 1996; Tian *et al.*, 2010; Lin *et al.*, 2011).

However, based on these results alone, it is not possible to determine how much dimer formation (or what fluence) is required to ensure the parasite dies on penetration. Studies have previously shown that worms are able to develop from cercariae exposed to sub-lethal fluences, although they did suffer from morphological mutations, sexual anomalies, and sterility (Mohammed, 1999). Oguma *et al.* (2001) found that approximately 700 dimers per DNA molecule were required to achieve a 1-$\log_{10}$ reduction in *E. coli*, although the genome is much smaller than that of *Schistosoma* spp (4.6Mb compared to 270Mb, respectively (Oguma *et al.*, 2001; LoVerde *et al.*, 2004)). Future studies could mechanically transform cercariae into schistosomula following UV exposure and the morphology could be examined to determine if they are able to survive *in vitro*. It is also important to note that at these fluences schistosome cercariae may still be able to penetrate the skin and a wider discussion would be required among health practitioners as to whether that is acceptable from a public health standpoint.

5.5.2 Repair potential

The CV values were high for some samples but a linear relationship with a reasonable goodness of fit ($R^2 > 0.7$) was established at all wavelengths when using the endpoint data, suggesting dimer formation

is proportional to fluence. However, the mean OD_{620} measurements were higher at $0\,\mathrm{mJ/cm^2}$ than at $10\,\mathrm{mJ/cm^2}$ and $25\,\mathrm{mJ/cm^2}$ at 285 nm and higher than $25\,\mathrm{mJ/cm^2}$ at 265 nm. This is possibly a result of background noise due to inadequate blocking or washing steps, resulting in non-specific binding of the antibodies to the well plate. Improper washing technique is a common source of background noise in ELISA data, which is likely to improve with practice, however noise interference at low fluences could also be reduced by increasing the concentration of *S. mansoni* DNA. Alternatively, it is possible that there is a true shoulder in the data resulting from repair mechanisms. Following UV exposure, samples were exposed to ambient light in the laboratory for up to one hour before DNA extraction took place which may have lead to the photorepair of some dimer sites.

Ruelas *et al.* (2007) found that there was a significantly higher prevalence of infection in *B. glabrata* snails that were maintained under bright fluorescent white light immediately after exposure to *S. mansoni* miracidia that were irradiated with a sub-lethal fluence of UV-B light (313 nm, $86.1\,\mathrm{mJ/cm^2}$) than in control snails that were maintained in the dark. This suggests *S. mansoni* miracidia are able to photoreactivate, although it is unclear how many of the miracidia became infective, as one miracidium may produce up to 600 secondary sporocysts (Ruelas *et al.*, 2007; Jamieson *et al.*, 2017). In contrast, there was no significant difference in the survival of *M. novaezealandensis* cercariae that were exposed to fluorescent white light following exposure to UV-A and UV-B light. In infectivity experiments in the same study there was a slight increase in the number of successful infections by the cercariae that were kept in the light conditions, but this was not statistically significant and the authors concluded that there was no evidence that *M. novaezealandensis* cercariae could photoreactivate (Studer *et al.*, 2012).

Studer *et al.* (2012) suggest that trematode cercariae are not able to repair UV damage because these mechanisms require considerable amounts of energy that would drain their limited glycogen reserves. Instead the parasite compensates for the vulnerability of cercariae by producing them in large numbers (Studer *et al.*, 2012). Whilst adult schistosome worms may produce hundreds or even thousands of eggs each day (Moore and Sandground, 1956; Ayad, 1974), only some of these will reach the environment and in each egg there is only one miracidium. Therefore the ability to photoreactivate during the snail infective life stage may be a wiser energy investment. That said, the photoreactivation capabilities of schistosome cercariae definitely warrants further investigation, particularly considering that human schistosome cercariae are phototactic and have evolved to shed from their snail hosts during the hottest and, therefore brightest, part of the day (Short and Gagné, 1975; Mouahid *et al.*, 1991; Combes *et al.*, 1994; McKerrow and Salter, 2002).

5.5.3 Wavelength dependence

In the previous chapter it was suggested that UV induced RNA damage could be preventing protein translation in cercariae, resulting in eventual death. There are no commercially available anitbodies that are specific to UV photoproducts in RNA, and RNA damage has therefore not been directly measured here. However as the absorption characteristics of DNA and RNA are very similar, it can be assumed that the production of cyclobutane pyrimidine dimers in the nucleic acids is also similar (Harm, 1980; Kowalski *et al.*, 2009). This would suggest that the fluences required to induce dimerization of RNA are also much lower than the fluences required to kill cercariae. However, considering RNA exists in many copies within a cell, it follows that higher fluences are required as multiple RNA molecules

need to be damaged to prevent the cell from functioning. This would suggest kinetics based on the multi-target theory discussed in Section 2.4.3. RNA damage could be directly measured by using alternative methods such as reverse transcript-qPCR, which has previously been used to study the UV disinfection mechanism in RNA viruses (Beck *et al.*, 2016). Whilst a benefit of this method is that it is not specific to any one photoproduct, it is based on a relatively short sequence of RNA (although multiple sequences could be tested) and it produces a decaying signal that saturates to zero, and therefore may not be suited to high fluence applications (Blyth *et al.*, 2021).

The most effective wavelength for inducing dimer formation was 265 nm which had the highest rate constant ($k = 0.0003$), however there was no statistical difference between the 265 nm and 255 nm rate constants ($k = 0.0002$). The 285 nm LEDs were least effective at inducing dimer formation, and the rate constant ($k = 0.0001$) was statistically different to both the 255 nm and 265 nm LEDs. Given the absorption spectra of DNA relative to the spectral power distribution for each of the UV LEDs (Figure 5.5), the wavelength dependence of dimer formation is demonstrated here is generally as expected. However, the microscopy results in the previous chapter found that the 265 nm and 285 nm LEDs were significantly more effective at killing cercariae than the 255 nm LEDS. Considered together, the results of the microscopy experiments and the ELISA suggest that a different UV disinfection mechanism may be dominant when schistosome cercariae are exposed to polychromatic UV light with peaks between 265 nm and 285 nm.
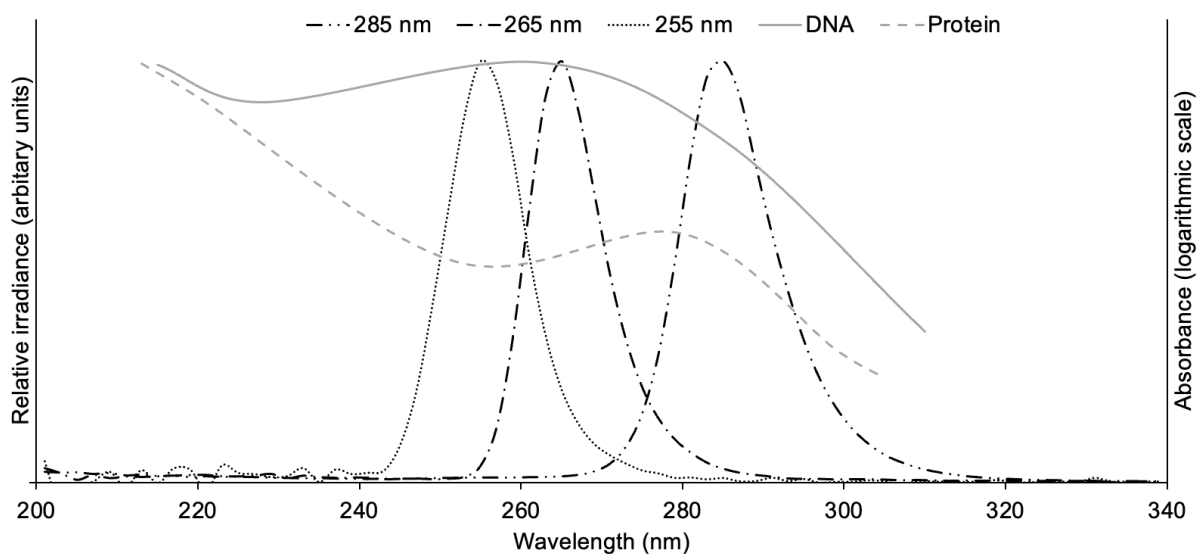
Figure 5.5 Spectral power distribution of the three ultraviolet light-emitting diodes and the relative absorption of DNA and protein.

A previous study investigating the inactivation of *M. novaezealandensis* cercariae in sunlight found that UV-A and UV-B exposure induced both DNA dimerization and oxidative stress which was evidenced by oxidation of proteins (Studer *et al.*, 2012). However as the cercariae in these experiments were exposed to filtered sunlight, the exact spectrum of light they were exposed to is unknown and it was not determined which mechanism was dominant. Exposure to UV light between 265 nm and 295 nm has been shown to produce intracellular reactive oxygen species in bacteria, with the amount of ROS increasing with wavelength (Pousty *et al.*, 2021). Given the absorption spectra of protein relative to the spectral power distribution of the LEDs its possible that protein absorption also contributed to the inactivation of cercariae (Figure 5.5). Both these disinfection mechanisms could explain why the

265 nm and 285 nm LEDs are more effective at killing schistosome cercariae than the 255 nm, which emit very little light over 270 nm.

Attempts were made to study protein damage in schistosome cercariae by examining the effect of UV light on the integrity of cell membranes, which is where the majority of UV absorbance by proteins occurs (Harm, 1980; Bolton and Cotton, 2008). UV exposed cercariae were dual-stained with a vital and non-vital dye, fluorescein diacetate (FDA) and propidium iodide (PI), respectively. FDA is a cell-permeable dye that is hydrolyzed to green fluorescein in living cells by the enzyme esterase. It is only retained in cells with intact cell membranes (Boyd *et al.*, 2008). PI is a red fluorescent DNA stain, which is non-cell-permeable and therefore will only stain DNA if there has been a breach in the cell membrane (Tawakoli *et al.*, 2013). This assay has previously been used to identify viable and non-viable schistosomula that have been exposed to anti-schistosomal compounds (Peak *et al.*, 2010) and schistosome cercariae that had been heat killed (Braun *et al.*, 2020a). PI has also been used to assess the integrity of *E. coli* cell membranes following UV disinfection and chlorination (Xu *et al.*, 2018). Preliminary experiments were conducted using the method devised by Braun *et al.* (2020a) and the results are shown in Figures C.1 and C.2 in the appendices. It was possible to selectively stain samples containing alive and dead cercariae, and with increasing fluence there was a decrease in FDA fluorescence and an increase in PI fluorescence at both 265 nm and 285 nm. However because of the very small sample volumes required to carry out the assay in a multiwell plate ($< 250 \, \mu L$) compared to the sample volumes required for the UV exposures (3.8 mL), and the fact that samples containing alive cercariae cannot be centrifuged as it may damage them and cause the head and tail to separate, it was very difficult to ensure an even number of cercariae per well. This lead to very high standard deviations in the data, particularly for the FDA fluorescence, and it was not possible to make any meaningful comparisons between the two wavelengths tested. Future experiments to assess protein damage could use protein precipitation and Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis, which separates intact proteins by molecular weight and has previously been usedto quantify UV damage in adenovirus proteins (Eischeid and Linden, 2011; Beck *et al.*, 2018).

5.5.4   Species

Whilst the absorption spectra of all nucleic acids have common features, such as a peak in absorbance between 260–265 nm, the base compositions vary between species. Therefore the exact absorbance spectrum and the number of potential dimer sites will also vary between species. The extent of dimerization may be predicted by genomic modelling which uses base counting software to determine the number of potential dimer sites in the DNA (Kowalski, 2011). The schistosome genomes are too large to be analysed using this method, which has so far only been tested on bacteria and viruses (Kowalski *et al.*, 2009; Kowalski, 2011). However as the AT content of the genomes of each of the main schistosomes is similar (*S. mansoni* 65.3%, *S. haematobium* 65.7%, *S. japonicum* 66.5%) it would be surprising if there were significant differences in cyclobutane pyrimidine dimer formation (Matallana-Surget *et al.*, 2008; Kowalski, 2011). That said, the susceptibility for dimerization is not just dependent on the AT content but also the number of potential dimer sites (i.e. adjacent pyrimidine bases), repair capabilities (if any), and other physiological differences (e.g. size, tegument thickness) that may effect the UV sensitivity of the organism (Kowalski *et al.*, 2009; Kowalski, 2011). Further research is therefore required to confirm if there is a difference in dimer formation in the other main human species, *S. haematobium* and *S. japonicum*.

5.5.5 Study limitations

The main limitation of this study is that, due to time constraints and difficulties obtaining laboratory consumables, the ELISA was only run once and the CV values between samples were high for some fluences. There are a number of possible causes of variation between wells in an ELISA, such as improper washing, inconsistent pipetting, or errors in sample preparation such as insufficient mixing of defrosted DNA samples. Two DNA samples were assayed in duplicate for each fluence (total of four measurements per fluence) and there is generally good agreement between duplicate measurements of the same DNA sample. This suggests there may have been inconsistencies in sample preparation. However, considering this was the first attempt, its possible that the high CV values could be a result of a combination of the issues described here and the experiments should be replicated to confirm the significance of the results.

5.6 Conclusions

Compared to bacterial and viral pathogens, there has been very little research into the UV disinfection mechanisms of complex multicellular microorganisms such as helminth eggs and larvae. Whilst there were limitations to the experiments, the work presented in this chapter explicitly demonstrates for the first time that cyclobutane pyrimidine dimers are formed in *S. mansoni* DNA when cercariae are exposed to UV light in the germicidal range. Dimers were formed when cercariae were exposed to $50\,\mathrm{mJ/cm^2}$ at all wavelengths, suggesting that *S. mansoni* cercariae may be made non-viable at much lower fluences than is required to kill them. However they may still be able to penetrate the skin, and results presented here can not confirm how many dimers are required to ensure the death of cercariae following penetration. Further research is therefore required to determine if fluences lower than those presented in the previous chapter are sufficient for making water safe from cercariae.

Exposure to the 255 nm and 265 nm LEDs resulted in significantly higher levels of dimer formation than exposure to the 285 nm LEDs. This is the opposite of the results presented in the previous chapter that found the 265 nm and 285 nm LEDs were more effective at killing *S. mansoni* cercariae. This suggests that nucleic acid damage may not be the only mechanism responsible for killing cercariae at these wavelengths. Possible mechanisms that warrant further investigation include protein absorption and oxidation of critical cellular components including nucleic acids, lipids, and proteins. The repair potential of *S. mansoni* cercariae was not investigated at this time, and this also warrants further study.

6 Discussion

Mass drug administration of praziquantel has undoubtedly been effective at reducing the global health burden of schistosomiasis (Fenwick *et al.*, 2009; Rollinson *et al.*, 2013) but drug donation programmes only reached 44% of the at-risk population in 2019 (WHO, 2020a) and treatment cannot prevent reinfection if contact with cercariae infested water continues. As a result, hotspots remain in communities that are reliant on surface water for daily domestic, recreational, or occupational activities. Current control strategies that focus on PC can only have a temporary effect on transmission (King, 2009; Gray *et al.*, 2010; Guidi *et al.*, 2010) and the reliance on a single form of treatment leaves PC campaigns vulnerable to both supply chain issues and drug resistance (Doenhoff *et al.*, 2002). It is now widely accepted that improvements in WASH infrastructure are also required if the disease is to be eliminated as a public health problem by 2030 (Utzinger *et al.*, 2003; Waite *et al.*, 2016; Lo *et al.*, 2017; Ross *et al.*, 2017; WHO, 2020b; Boisson *et al.*, 2021). Schistosomiasis is contracted by skin contact with fresh water that contains cercariae. Whilst completely preventing water contact in endemic regions is unrealistic (Secor, 2014), providing access to improved water sources can reduce both the frequency and duration of contact with fresh water bodies (Farooq *et al.*, 1966; Lima e Costa *et al.*, 1987; Wepnje *et al.*, 2019) and piped water supplies have been shown to reduce the prevalence, incidence, and intensity of schistosomiasis infections in many locations (Jordan *et al.*, 1975; Mason *et al.*, 1986; Sarvel *et al.*, 2011; Ebai *et al.*, 2016; Tanser *et al.*, 2018; Mogeni *et al.*, 2020). Where piped water is not available, household and community scale water treatment processes can be used to treat water that has been collected from contaminated surface water bodies, reducing the risk of infection.

The emerging technology of UV LEDs has the potential revolutionise water disinfection, providing mercury- and chemical-free water treatment that is suitable for remote and low-income regions (Ibrahim *et al.*, 2014; Lui *et al.*, 2014; Loeb *et al.*, 2016; Linden *et al.*, 2018; Linden *et al.*, 2019). There are a range of ways that SODIS or UV disinfection could be harnessed for schistosomiasis control programs, from providing SODIS buckets for doing laundry (Polo-López *et al.*, 2019) to supporting local entrepreneurs to build water kiosks (Opryszko *et al.*, 2013). However, there are currently no formal guidelines for using UV disinfection (or any other water treatment processes) against schistosome cercariae, making it difficult to implement WASH programs that directly target schistosomiasis transmission (Braun *et al.*, 2018; Campbell *et al.*, 2018). The overall aim of this research was therefore to investigate the effectiveness of UV disinfection against schistosome cercariae in water and examine the possible mechanisms involved.

The thesis started with a systematic review of the UV sensitivity of schistosomes and other WASH-related helminths in Chapter 3. There was very little published research into the effectiveness of UV against helminth eggs and larvae and the understanding of the UV disinfection mechanisms was limited, particularly when compared to bacteria and viruses which have been widely studied (Malayeri *et al.*, 2016). Schistosome cercariae appeared to be more sensitive to UV than other helminths that remain as an egg in the environment, such as ascaris (Tromba, 1978; Lucio-Forster *et al.*, 2005; Brownell and Nelson, 2006), however different methods were used to assess viability. Almost all the data for schistosome cercariae were from vaccination studies that were focused on the immune response

of the animal host, rather than the direct effect of UV light on cercariae. The review found that approximately 5–$14\,\text{mJ/cm}^2$ at $253.7\,\text{nm}$ was required to achieve a 1-$\log_{10}$ reduction in schistosome worm burden (Dean et al., 1983; Fang-Li et al., 1996; Kamiya et al., 1993; Kumagai et al., 1992; Lin et al., 2011; Tian et al., 2010; Wang et al., 1993), but at these fluences UV light had no visible effect on the cercariae, which were still able to locate and infect the animal hosts. It was unclear if cercariae were prevented from developing into adult worms as a direct result of UV damage to the parasite or as a result of an enhanced immune response to irradiated cercariae (Mohammed, 1999; Kusel et al., 1989). It was also not clear at what point in the development the parasite died. One study demonstrated that short exposure to UV light resulted in a significant increase in mortality of S. mansoni cercariae during skin penetration (Ghandour and Webbe, 1975), whilst another found that they were able to develop into adult worms, although they did suffer from from mutations and sexual anomalies (Mohammed, 1999). The fluence was not recorded in either study. It was concluded that whilst the published studies suggested schistosome cercariae may be relatively sensitive to UV light, the data were insufficient for producing detailed recommendations for water treatment purposes, and further experimental research was required.

To address some of these knowledge gaps, in Chapter 4 the UV fluence-response of S. mansoni cercariae was determined at four peak wavelengths in the germicidal range. The direct effect of UV on cercariae was investigated by examining their morphology and motility under the microscope to determine if they were alive or dead. This was the first reproducible study to determine the fluence-response of S. mansoni cercariae. It was shown that the fluences required to kill S. mansoni cercariae were much higher than the immunisation studies reviewed in Chapter 3 suggested were required to prevent development into adult worms. At the most effective wavelength, $265\,\text{nm}$, a fluence of $247\,\text{mJ/cm}^2$ was required to achieve a 1-$\log_{10}$ reduction in alive S. mansoni cercariae. Similar differences have been observed in other helminths such as Ascaris suum, for which the fluence required to achieve a 1-$\log_{10}$ reduction in viable embryos was approximately 10 times higher than the fluence required the achieve the same reduction in worm burden (Brownell and Nelson, 2006; Tromba, 1978). The difference between these studies was attributed to a delay in the expression of UV-induced DNA damage during cell replication (Brownell and Nelson, 2006), but schistosome cercariae do not undergo any DNA replication or transcription until they transform into schistosomula (Roquis et al., 2015). It was therefore suggested that any DNA damage which may be induce at lower fluences and which may prevent development into adult worms will not become evident until after cercariae have penetrated the skin. It was also concluded in Chapter 4 that DNA damage was unlikely to be responsible for the visible damage or death of cercariae before penetration and alternative mechanisms, such as RNA damage, protein damage, and oxidative stress, were discussed. Given the cercariae were exposed to polychromatic light (excluding samples exposed to the LP lamp), it was suggested that multiple UV disinfection mechanisms may have contribute to the visible damage and death of cercariae.

Due to the energy requirements and cost implications, the fluences required to immediately kill cercariae were considered too high for UV disinfection to be a suitable water treatment method for low-income regions, at present. It was therefore necessary to investigate if DNA damage was induced by UV exposure, as this would help to understand if cercariae can be made non-viable at lower fluences and the mechanism involved. In Chapter 5 an ELISA was used to detect cyclobutane pyrimidine dimers in the DNA of UV exposed S. mansoni cercariae. The experiments demonstrated that, at all wavelengths studied, fluences as low as $50\,\text{mJ/cm}^2$ were able to induce cyclobutane pyrimidine dimer formation,

and at the most effective wavelengths, 255 nm and 265 nm, dimers were formed at even lower fluences. This confirmed that, even though cercariae may not be visibly damaged, exposure to low UV fluences does induced damage on a molecular level. If a sufficient number of dimers are formed, DNA replication and transcription will stall (Harm, 1980; Oguma *et al.*, 2001), resulting in the death of some cells and possibly the death of the parasite. However this damage will not become evident until after cercariae have transformed into schistosomula, when these processes resume (Roquis *et al.*, 2015). This explains the differences between the results of systematic review and the fluence-response experiments, and suggests that cercariae may be non-viable even if they are not visibly damaged. However, it was not with the scope of the research to determine what amount of dimerization was required to ensure that cercariae will die upon skin penetration. Further research is required to confirm if schistosome cercariae are non-infective or non-viable at fluences lower than those presented in Chapter 4. Until then a conservative approach based on the total death of cercariae is appropriate for developing water treatment guidelines.

6.1 Implementing the research outcomes in practice

The high fluences required to kill *S. mansoni* cercariae indicate that UV disinfection may not currently be an energy- or cost-efficient water treatment method for providing water for contact activities that is immediately free of schistosome cercariae, particularly compared to the relatively low contact times required to achieve similar levels of disinfection using chlorination (Braun *et al.*, 2020b). Small scale UV LED water treatment systems that have been validated to international standards are currently commercially available for approximately 100–2000 USD depending on required flow rates and level of treatment (Lear, 2021). An example point-of-use system is capable of delivering a fluence of $40 \, \text{mJ/cm}^2$ at a flow rate of 2.25 L per minute, whereas larger and more expensive systems can deliver $40 \, \text{mJ/cm}^2$ at a flow rate of 45 L per minute, under optimal conditions (Aquisense Technologies, 2021). This is considerably less than is required to kill schistosome cercariae, although pilot studies have demonstrated that higher fluences in the order of $120 \, \text{mJ/cm}^2$ can be achieved at much lower flow rates (Hull *et al.*, 2019). The current capital cost of UV LED water treatment systems are likely too high for use in WASH programmes, particularly those targeting schistosomiasis transmission. However, improvements in wall plug efficiencies combined with cheaper production costs may make UV LED technology more competitive, in terms of the whole life energy and cost requirements, in the near future (Ibrahim *et al.*, 2014; Zhang *et al.*, 2019; Kneissl *et al.*, 2019; Krames, 2020). Only if such predictions are realised and future studies are able to confirm schistosome cercariae are made non-infective or non-viable at lower fluences, will UV LED disinfection become a realistic option for treating water for contact activities to protect against schistosomiasis transmission.

Whilst UV LEDs may not currently be a feasible solution for water treatment in low-income regions, their potential should not be ignored. Their small size and robustness means UV LEDs are perfect for innovative WASH solutions such as a "zero-energy" UV LED hand pump (Sundar and Kanmani, 2020). Point-of-use systems could be fitted into taps at laundry and shower stations, and as UV disinfection is effective against a wide range of water-borne bacteria, viruses, and protozoa, they could also be used to treat drinking water with no risk of over-dosing or production of disinfection by-products. UV disinfected water may also be more acceptable to people who are sensitive to the taste and smell of chlorine (Jeuland *et al.*, 2016; Crider *et al.*, 2018; Smith *et al.*, 2021). Current UV-LEDs have

an operating life of 5,000–10,0000 hours, which is about 6–12 months continuous operation, however because there is no warm up time they only need to be switched on as needed, when water is flowing. A pilot study in the USA found a combined sand filter and UV-LED disinfection system could be run for a year, without any maintenance and without any reduction in effectiveness, for an annual operating cost of less than 25 USD (Hull *et al.*, 2019).

Conventional UV technology utilising LP mercury arc lamps is already being used to provide household and community scale water infrastructure in low-income regions. Whilst these systems may not have all the advantages of UV LEDs, they have considerably lower capital costs, with simple household scale systems costing less than 50 USD (Brownell *et al.*, 2007; Reygadas *et al.*, 2018). Small water enterprises using a combined treatment of filtration and UV disinfection have been shown to be a sustainable and effective approach to providing community scale water treatment (Hunter *et al.*, 2013; Opryszko *et al.*, 2013). Furthermore, LP mercury arc lamps have considerably higher output power and wall plug ratios than UV LEDs, meaning fewer lamps and shorter exposure times would be required to reach the high fluences required to kill schistosome cercariae. However, the LP mercury arc lamp was the least effective at killing schistosome cercariae, with a 1-$\log_{10}$ reduction only achieved after storing the samples for 3 hours at the fluences studied. Further research is required to determine the fluence needed to immediately kill schistosome cercariae at this wavelength, or to confirm if they are made non-viable at lower fluences, before any solution can be implemented using LP mercury arc lamps.

6.2 Future research recommendations

This research rigorously examined the effectiveness of UV disinfection at killing *S. mansoni* cercariae. Experiments should be repeated with *S. haematobium* and *S. japonicum* cercariae, which although morphologically similar, may exhibit different sensitivities to UV light due to genomic and behavioural differences (e.g. *S. japonicum* cercariae tend to cling to the water surface (He *et al.*, 2005)). It was originally intended that the UV sensitivity of both *S. mansoni* and *S. haematobium* cercariae would be examined in this thesis, however there was difficulty obtaining infected *Bulinus* spp. snails in the UK.

The experiments presented in this thesis were carried out under controlled laboratory conditions using lab-reared *S. mansoni* cercariae. To confirm the results are valid for use in the field, they should be repeated using "wild type" cercariae collected from regions endemic to schistosomiasis. Under my supervision, preliminary field experiments were carried out by MSc student Eden Eritrea at AAU (Eritrea, 2021). The experiments were carried out using a similar method to that presented in Chapter 4 on schistosome cercariae that were shed from snails collected in Ethiopia from the banks of Lake Ziway and the Tikur Wiha River near Hawassa, approximately 167 km and 279 km south of Addis Ababa, respectively. The preliminary results were generally in agreement with those present here. The 285 nm LEDs were found to be slightly more effective than the 265 nm LEDs, however each wavelength was only tested once so it is unclear if this difference is statistically significant. The snails shed a number of species of schistosome cercariae including *S. mansoni* and several avian species. It was not possible to separate these, so they were analysed together. The experiments should be repeated using water samples containing only *S. mansoni* cercariae and compared to the results presented here.

Future experiments should also examine the infectivity and viability of schistosome cercariae following

exposure to lower UV fluences. Infectivity could be examined by exposing cercariae to human skin samples obtained from cosmetic surgery and calculating the difference between the number of cercariae in the sample before and after exposure to the skin (Bartlett *et al.*, 2000). Careful consideration should be given to parameters such as the proximity of the cercariae to the skin sample and the temperature of the skin. The use of mice tails instead of human skin could also be used to examine infectivity. Mice tails are often a waste product of scientific research that requires animal testing, and may be more readily available than human skin samples. This method is being developed by current PhD student Jiaodi Zhang.

To assess the viability of schistosome cercariae following UV exposure, cercariae could be mechanically transformed into schistosomula by repeated passage through a 22 gauge needle and incubation at 37°C and 5% CO_2 in tissue culture medium (Colley and Wikel, 1974). Schistosomula morphology could be examined under the microscope at repeated intervals over 24 hours to determine if they are still alive or the fluorescence assay briefly described in Chapter 5 could be used. This assay has already been successfully used to identify viable and non-viable schistosomula that have been exposed to anti-schistosomal compounds (Peak *et al.*, 2010). Post-transformation, DNA transcription should recommence within 3–4 hours in viable schistosomula and cell replication with 18 hours (Roquis *et al.*, 2015), therefore studying schistosomula over 24 hours should be sufficient to determine viability. It is expected that non-viable schistosomula that have a formed a critical number of dimers due to UV exposure would not be able to complete these processes, resulting in cell death and ultimately death of the schistosomula.

This research also investigated the possible UV disinfection mechanisms in schistosome cercariae by examining wavelength dependent dimer formation in *S. mansoni* DNA. Due to time constraints and difficulties obtaining laboratory consumable the ELISA was only run once and there were high CV values for some samples. The experiment should therefore be replicated to confirm the significance of the results. The photoreactivation potential of schistosome DNA should also be examined by comparing the difference in dimerzation between samples that are exposed to fluorescent white light and samples that are kept in the dark, immediately after UV exposure. It would also be interesting to repeat these experiments with the other main human schistosomes, as dimerization potential is a function of the microorganisms genome. To further improve the understanding of UV disinfection mechanisms in large, multicellular microorganisms such as schistosome cercariae, experiments should be carried out to investigate the wavelength dependence of different mechanisms. Oxidative stress and protein damage could be analysed using the methods previously described by Studer *et al.* (2012) and (Beck *et al.*, 2018) respectively.

SODIS is a low cost water treatment method that should be investigated in more detail. Experiments should be conducted in endemic regions, using large volumes of water and appropriate reactors, such as 20 L transparent polypropylene buckets, which have been shown to be as effective as PET drinking bottles for inactivating bacteria and protozoa (Polo-López *et al.*, 2019) and would be well suited for water contact activities such as hand washing, bathing, and laundry. Initially experiments should be carried out in controlled conditions, using schistosome cercariae that are spiked into bottled mineral water. The reactors should then be exposed to sunlight for different lengths of time in a variety of conditions (e.g. full sunlight, overcast) and at different times of the day to determine the minimum exposure time to inactivate cercariae. For drinking water, the minimum recommended exposure time

is 6 hours when using a 2 L PET bottle in full sunlight (Luzi *et al.*, 2016). The lifespan of cercariae decreases with water temperature and at 40–45 °C it is less than 6 hours (Lawson and Wilson, 1980; Braun *et al.*, 2018). These water temperatures have been exceeded in a number of SODIS reactors (Lawand *et al.*, 1997; Keogh *et al.*, 2015; Polo-López *et al.*, 2019), but will depend on the reactor, the volume of water used, and the weather conditions. On overcast days the minimum recommended exposure time for drinking water is 48 hours, after this time it is likely that all cercariae will have died as a result of glycogen depletion (Lawson and Wilson, 1980). Following sunlight exposure the water could be filtered through a Pitchford funnel to concentrate the cercariae in a smaller volume of water (Pitchford and Visser, 1975), cercariae may get stuck to the sides of the funnel so care should be taken to thoroughly rinse the filter material. The motility and morphology could be assessed under a microscope to determine if cercariae are alive or dead, or infectivity or viability experiments could be conducted as described above. The ELISA assay presented in Chapter 5 could also be used to investigate the SODIS disinfection mechanisms in more detail.

Water samples with high organic matter content or high turbidity will absorb UV light and particles may shield cercariae, therefore the impact of water quality on the effectiveness of UV and solar disinfection against schistosome cercariae should be investigated and the experiments described here should be repeated using water samples collected from endemic regions. For water of particularly poor quality the effectiveness of UV disinfection may be improved by a pre-treatment process such as filtration or sedimentation, which often proceed UV disinfection in water treatment plants. It is very likely that filtration will reduce the concentration of cercariae (Braun *et al.*, 2021), therefore lower fluences may be sufficient to inactivate all cercariae in the water. The effectiveness of combined water treatment processes at removing and inactivating cercariae should be investigated in more detail.

6.3   Towards integrated schistosomiasis control

Whilst UV disinfection may not currently be a feasible solution for providing safe water for contact activities, recent studies have shown other water treatment processes such as chlorination and filtration are effective against schistosome cercariae (Braun *et al.*, 2020b; Braun *et al.*, 2021). There is now sufficient evidence to begin to develop water treatment guidelines for WASH programmes targeting schistosomiasis. Implementing these programmes will require extensive collaboration between the NTD and WASH communities and engagement of specialists from a number of disciplines including engineers and WASH practitioners, social scientists, parasitologists, and economists (Boisson *et al.*, 2021). Most importantly early consultation with local communities is essential to ensure the successful implementation of any WASH programmes (Niederberger and Glanville-Wallis, 2019; Nelson *et al.*, 2021; Tsekleves *et al.*, 2022). Providing facilities for domestic and recreational water contact activities, such as laundry stations, may improve the effectiveness of water treatment interventions (Taylor *et al.*, 1989; Noda *et al.*, 1997; Kosinski *et al.*, 2012), however local socio-cultural factors, such as the need for privacy or preference for certain bathing sites, need to be taken into account to ensure they truly meet the needs of the community. Consideration also needs to be given to ownership and operational and maintenance requirements to ensure continued use (Montgomery *et al.*, 2009; Marks *et al.*, 2013; Foster *et al.*, 2018; Contzen and Marks, 2018). Long term health education campaigns should be implemented to help maintain compliance (Patel *et al.*, 2012; Joshi and Amadi, 2013; Reygadas *et al.*, 2018). Such campaigns should be timed to coincide with rounds of PC to improve treatment coverage and reduce

the risk of reinfection (Mwanga and Lwambo, 2013; Inobaya *et al.*, 2018).

It is not feasible to completely eliminate surface water contact by providing safe alternatives for domestic activities such as laundry and bathing, particularly as many at-risk communities are reliant on fresh water bodies for income from fishing and other occupations (Secor, 2014). However, studies have shown that high coverage of access to safe water reduced the overall risk of infection within the community, as indirect protection is provided to people who continue to frequently use surface water bodies by a reduction in the overall reservoir of schistosomes (Tanser *et al.*, 2018). Additional interventions such as providing boots or waders for people working on the shores of lakes and rivers may help to further reduce exposure from occupational activities, and targeted snail control may also be effective. Although careful consideration and consultation with the community is vital before implementing any intervention that may effect the local ecosystem, particularly in communities that are reliant on fishing.

Schistosomiasis is a disease of poverty which cannot be eliminated until everyone has access to the basic resources they need to lead healthy lives, including WASH infrastructure. Whilst there are barriers to the widespread integration of NTD and WASH interventions (Freeman *et al.*, 2013; Johnston *et al.*, 2015; Waite *et al.*, 2016), recent developments in national programming in countries across Africa and South East Asia have demonstrated that it is achievable (Khieu *et al.*, 2019; WHO, 2020b; Boisson *et al.*, 2021).

7 Conclusions

Schistosomiasis is a water-based neglected tropical disease that is transmitted to humans via skin contact with water containing cercariae, the human infective stage of the *Schistosoma* parasite. Providing access to safe water has been shown to reduce transmission of schistosomiasis by minimising contact with cercariae in infested surface water bodies (Jordan *et al.*, 1975; Jordan *et al.*, 1982; Mason *et al.*, 1986; Sarvel *et al.*, 2011; Ebai *et al.*, 2016; Tanser *et al.*, 2018; Mogeni *et al.*, 2020). Water treatment processes can be used to kill or inactivate cercariae and making water safe for contact activities such as laundry and bathing. UV LEDs are an emerging technology that have the potential to revolutionise water treatment infrastructure in low-income regions (Ibrahim *et al.*, 2014; Lui *et al.*, 2014; Loeb *et al.*, 2016; Linden *et al.*, 2019). The overall aim of this thesis was to determine the effectiveness of UV disinfection against schistosome cercariae and examine the photobiological mechanisms involved. This was achieved by addressing three objectives, the results of which are summarised below.

1. Systematic review

   - Review existing literature on UV sensitivity of WASH-related helminths, including schistosome cercariae. What helminths have been studied? What UV wavelengths have been tested? What methods were used to establish viability and are they comparable?

   - Determine which helminths may be more susceptible to this form of water treatment, based on available studies. Do the data suggest schistosome cercariae are susceptible to UV disinfection? Are the data reliable?

   - Identify gaps in research which will inform future studies regarding the proper use of UV and SODIS for minimising the spread of these diseases via water in low-income regions.

   Outcome: The systematic review revealed that there had been little research into the effectiveness of UV disinfection against helminth eggs and larvae. Most studies used LP mercury lamps, although some studies investigated the effectiveness of SODIS and UV-A and UV-B light. Methods for assessing viability varied, even between papers studying the same species, resulting in large ranges in the fluence-response. The direct effect of UV light on schistosome cercariae was not quantified, but relatively low fluences (5–$14\,\mathrm{mJ/cm^2}$ at $253.7\,\mathrm{nm}$) were required to achieve a 1-$\log_{10}$ reduction in worm burden. At these fluences cercariae were visibly unharmed and able to penetrate skin. Only one study used a published protocol to calculate fluence (Brownell and Nelson, 2006), all other fluences should therefore be considered approximate. There was evidence to suggest that UV disinfection may be effective against schistosome cercariae and other helminths, but there were insufficient data to produce detailed UV disinfection guidelines.

2. UV fluence-response

   - Examine the direct effect of UV disinfection on *S. mansoni* cercariae in water, using established protocols for fluence determination.

   - Determine the fluence-response at each wavelength. How does this compare to the literature?

- Which wavelength is most effective? What does this suggest about the inactivation mechanism?

Outcome: *S. mansoni* cercariae were exposed to a range of fluences at four peak wavelengths across the germicidal spectrum. Published protocols (Bolton and Linden, 2003; Kheyrandish *et al.*, 2018) were used for fluence determination and the direct effect of UV light on cercariae was assessed at 0, 60, and 180 minutes post-exposure. The morphology and motility of cercariae were studied under the microscope to determine if they were alive and dead. UV LEDs emitting at a peak wavelength of 265 nm were found to be the most effective at killing *S. mansoni* cercariae, requiring a fluence of $247\,\mathrm{mJ/cm^2}$ to achieve a 1-$\log_{10}$ reduction. Cercariae were visibly damaged at lower fluences and the fluence required to achieve a 1-$\log_{10}$ reduction reduced to $127\,\mathrm{nm}$ and $99\,\mathrm{mJ/cm^2}$ if cercariae were stored for 1 and 3 hours, respectively. There was no statistical difference between UV LEDs at 265 nm and 285 nm one hour after exposure. The least effective wavelengths were 255 nm (UV LED) and 253.7 nm (LP lamp). The fluences required to kill schistosome cercariae were considerably higher than those reported in the literature to prevent cercariae from developing into adult worms. This is because cercariae do not undergo DNA replication or transcription until they transform into schistosomula upon skin penetration (Roquis *et al.*, 2015), therefore any DNA damage is not expressed until later in the life cycle when these processes resume. Possible disinfection mechanisms responsible for killing cercariae were discussed including RNA damage, protein damage, and oxidative stress.

3. UV damage mechanism

- Investigate possible UV damage mechanisms using immunological assays to assess UV-induced DNA damage in *S. mansoni* cercariae.
- Can UV-induced DNA damage be measured in schistosome cercariae? Is it possible to determine a fluence-response?
- Does the fluence-response vary with wavelength? What does this demonstrate about the UV damage mechanisms in schistosome cercariae?

Outcome: Cyclobutane pyrimidine dimers were detected in *S. mansoni* DNA using an ELISA and a linear fluence-response was established at three peak wavelengths across the germicidal range, with a reasonable goodness of fit. Dimer formation was induced at $50\,\mathrm{mJ/cm^2}$ at all the wavelengths studied. The most effective wavelength was 265 nm although there was no statistical difference with dimer formation at 255 nm. Significantly fewer dimers were formed at 285 nm. The wavelength dependent dimer formation in *S. mansoni* DNA was as expected given the absorbance spectrum of DNA and the SPD of the UV LEDs used in this study. The results suggest nucleic acid damage may not be the primary disinfection mechanism responsible for killing cercariae at 265 nm and 285 nm, although it may make them non-viable at lower fluences. Further research is required to confirm this, until then a conservative approach based on the total death of cercariae is appropriate for developing water treatment guidelines, from a public health standpoint.

This thesis has addressed gaps in the knowledge of UV disinfection against schistosome cercariae. The high fluences required to kill *S. mansoni* cercariae and the high capital costs of UV LEDs suggests that UV LED disinfection is not currently a feasible solution for schistosomiasis control. However, if future studies confirm schistosome cercariae are made non-viable at lower fluences and predicted

improvements in UV LED technology are realised, it may become a realistic option in the near future. As countries look toward eliminating schistosomiasis as a public health problem, innovative solutions will be required to improve access to WASH infrastructure and accelerate preventive chemotherapy campaigns. Hopefully this thesis provides a starting point for delivering these solutions.

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Appendix A

A.1 Included and excluded papers

Table A.1 Summary of data extracted from studies included in the systematic review (where available).

General information	Helminth	Experimental condition	Exposure
Author	Species	Maximum water temperature (°C)	Fluence (mJ/cm^2)
Date	Host	Water characteristic	Time (mins)
Title	Life stage	Lamp type	
Language	# organisms	Wavelength (nm)	
	Viability assessment	Distance (mm)	

Table A.2 List of papers included in the systematic review and some comments on the studies.

Reference	Title	Comments
Roh, 1968	The influence of ultraviolet irradiation upon the development and infectivity of hookworm larvae	Noted a reduction in egg production pathogenicity in worms developed from irradiated groups compared to control. Also carried out experiments with UV lamp (type unknown) on eggs about found less than 50% of eggs were able to develop/hatch when exposed for 1 hour 100mm from source.
Spindler, 1940	Effect of tropical sunlight on eggs of *Ascaris suis* (Nematoda), the large intestinal roundworm of swine	Max log reduction assumes all 5,000 eggs were counted in the experiment. Separate experiments found dry single cell eggs were less resistant however embryonated eggs were more resistant.
Jones and Hollaender, 1944	Effect of long ultraviolet and near visible radiation on the eggs of the nematodes *Enterobius vermicularis* and *Ascaris lumbricoides*	Lamp type chosen to simulate sunlight. Eggs were kept in single layers but there was an issue of "clumping" in some experiments. Eggs in later stages demonstrated no greater resistance but these results are considered less reliable because viability assessment method. Dry eggs were slightly less resistant. Notes that sunlight would be expected to be more damaging that the mercury source used for this experiment due to the presence of infrared radiation and higher temperatures.
Shikhobalova and Gorodilova, 1944	The effect of the ultraviolet part of the sun spectrum on the eggs of Ascaris.	Eggs were exposed to intermittent sunlight over a period of days, yellow filters were used to block UV light but allow visible light to penetrate. All eggs without filter died after a total exposure of about 60 hours. Over 90% of eggs with yellow filters were able to survive over 130 hours.
Miretski, 1956	Influence of natural ultra-violet radiation on *Ascaris lumbricoides* eggs.	Eggs were exposed to intermittent sunlight over a period of days, filters were used to transmit only UV, visible or infra red light. There was no significant difference between exposed eggs and controls. Suggests yellow-brown colour shell provide protection against sunlight.
Nenow, 1960	The Effect of Sunlight upon the Eggs of *Ascaris lumbricoides* at Different Altitudes.	Eggs were placed in direct sunlight at different altitudes, more eggs were inactivated at higher altitudes. Infective eggs were more resistant than single cell eggs.
Heaselgrave, 2011	The efficacy of simulated solar disinfection (SODIS) against *Ascaris, Giardia, Acanthamoeba, Naegleria, Entamoeba* and *Cryptosporidium*	Following exposure for 2 more hours no further inactivation was observed. Resistance to sunlight attributed to multi-layer egg shell.
Krakower, 1940	Some observations on the effects of physical and chemical agents on the cercariae of *Schistosoma mansoni*	1 hour exposure to sunlight killed most cercariae even when cloudy. 45 minute exposure to alpine lamp killed all cercariae.
Prah and James, 1977	Influence of physical factors on survival and infectivity of miracidia of *Schistosoma-mansoni* and *Schistosoma-haematobium* .1. Effect of temperature and UV light	Used distilled water which is known to inactivate cercariae. Also carried out experiments using mercury lamp (254nm) and 50-60 miracidia in 10ml dechlorinated tap water. Reduction in motility occurred after 30 seconds exposure, after 105 seconds most were motionless. Exposure for 60 or more prevented miracidia from penetrating snails.

Menon and Bhopale, 1985	Efficacy of UV-irradiated larval vaccine of *Ancylostoma ceylanicum* (Looss, 1911) in golden hamsters (*Mesocricetus auratus*)	Larvae became visibly sluggish after 1 minutes exposure and lost motility after 30-60 minutes. Hamsters were infected orally with 100 larvae. A minimum of 30 mins exposure was required for no worms to be recovered.
Kamath *et al.*, 1986	Effect of UV-irradiation, spleen cells and MLN cells on protective immunity against *Angiostrongylus cantonensis* infection in mice	Petri dish was agitated during exposures, no larvae died during exposures. Mice were orally inoculated, assumed larvae died during migration as none were found in brain tissue.
Ruelas, 2007	Sublethal effects of ultraviolet B radiation on miracidia and sporocysts of *Schistosoma mansoni*: intramolluscan development, infectivity, and photoreactivation	Decrease in presence of daughter sporocysts in snails at 30 mJ/cm². 86.1mJ/cm² prevented patent infection but miracidia were still able to penetrate snails. 300mJ/cm² is required to prevent penetration completely.
Vieira and Rombert, 1974	Study of immunity in ancylostomiasis II. Tentative immunization of the dog by inoculation of larvae of *Ancylostoma caninum* attenuated by ultraviolet irradiation	Mainly about vaccine. Larvae were checked on successive days after exposure, no larvae exposed to UV for 5 minutes at 40cm were able to survive 5 days or more.
Tromba, 1978	Effect of ultraviolet radiation on the infective stages of *Ascaris suum* and *Stephanurus dentatus* with a comparison of the relative susceptibilities of some parasitic nematodes to ultraviolet	Suspensions were gently mixed during exposures; some larvae were able to migrate to small intestines at 24mJ/cm² but about 48mJ/cm² migration was limited to lungs. Notes sensitivity is dependent on viability assessment as much higher fluence is required for inactivation in vitro than in host.
Aladawi *et al.*, 2005	Accelerated larvae development of *Ascaris lumbricoides* eggs with ultraviolet radiation	Results suggest exposure to UV increased larvae development.
de Lemos Chernicharo *et al.*, 2005	Inactivation of *E. coli* and helminth eggs in aerobic and anaerobic effluents using UV radiation	Flow through reactor, unclear how fluence was determined. High amount of suspended solids. Only fully developed, motile larvae are considered to be viable after 28 days incubation, inactivation may therefore be an over estimation when compared with other studies that assumed eggs in any stage of development were viable
Lucio-Forster *et al.*, 2005	Inactivation of *Ascaris suum* eggs by exposure to ultraviolet (UV) irradiation	Short paper, conference proceedings. Most correction factors are applied to the fluence in accordance with well-established protocol. Also used decorticated eggs and found they were more sensitive to UV.
Brownell and Nelson, 2005	Inactivation of single-celled *Ascaris suum* eggs by low-pressure UV radiation	Only 300 eggs from each sample were counted which limits the max log reduction that can be achieved. Used all correction factors in accordance with established protocol. Also carried out experiments with decorticated eggs found and found they were more sensitive to UV.
Mun, 2009	Inactivation of *Ascaris* eggs in soil by microwave treatment compared to UV and ozone treatment	Only 300 eggs from each sample were counted, limiting the max log reduction that can be achieved. Much higher fluences are required than in other studies, unclear why but the method for calculating the fluence has not been stated although does reference a study by Huffman et al. 2002 which makes some corrections.
Riwidiharso and Billalodin, 2006	Effect of ultraviolet irradiation (254 nm) on weakening ability of infecting miracidium *Fasciola gigantica*	Not much information about exposure methods. A minimum of 60 seconds exposure was required to significantly reduce the number of cercariae produced by snails infected with one irradiated miracidium.
McGavock and Howard, 1980	Effects of ultraviolet light on *Hymenolepis diminuta* ova and cysticercoids.	Ova were also tested in a separate experiment and found to be more sensitive than cystercoids
Standen and Fuller, 1959	Ultra-violet irradiation of the cercariae of *Schistosoma mansoni*. Inhibition of development to the adult stage	Very short exposure time so percentage error of exposure times likely to be high. Also examined motility and found 4 mins exposure at 2 cm from source was sufficient to kill 100% of cercariae. Also used a flow through reactor and achieved 0% worm recovery after exposure for 8 seconds.
Ghandour and Webbe, 1975	Effect of ultraviolet-radiation on cercariae of *Schistosoma-mansoni* and *Schistosoma-haematobium*	A series of experiments to investigate effect of UV on motility/behaviour, infectivity and viability. Physical effects on cercariae were recorded after 30-60 sec exposure, 5-20 secs had no visible effect. Very few exposed cercariae were able to migrate to the lungs in comparison to normal cercariae. No adult worms were recovered from cercariae exposed for 10 secs. UV also effected ability of cercariae to penetrate skin.

Rombert and Vieira, 1977	Irradiation of cercariae of *Schistosoma mansoni* with ultraviolet rays. I. Technic and results.	Mainly about vaccine. Results suggest male cercariae are more resistance to UV than female, as there were significantly more male worms derived from irradiated cercariae than female.
Dean, 1983	Immunization of mice with ultraviolet-irradiated *Schistosoma mansoni* cercariae: A re-evaluation	Worms that were able to survive exposure for 2 mins were "stunted". None were able to form fertile pairs as no pair worms or eggs were found.
Kusel *et al.*, 1989	Effects of irradiation and tunicamycin on the surface of glycoproteins of *S. mansoni*	Describes the effect of UV on the surface of cercariae. Irradiation causes the glycocalyx to be retained for longer after transformation to schistosomulae and caused changes in the structure of surface carbohydrates. Prevents synthesis of glycoproteins, inhibition of these for ~24hrs is sufficient to prevent development. Antibodies from host bond with higher affinity to schistosomula from irradiated cercariae.
Shi *et al.*, 1990	*Schistosoma japonicum*: An ultraviolet-attenuated cercarial vaccine applicable in the field for water buffaloes	Primarily about vaccine. Average of 3 worms recovered from pigs exposed to UV attenuated cercariae, average of 110 worms recovered from pigs exposed to 1000 normal cercariae
Ruppel *et al.*, 1990	*Schistosoma mansoni* and *S. japonicum*: Comparison of levels of ultraviolet irradiation for vaccination of mice with cercariae	Primarily about vaccine. 3 mJ/cm² reduced survival of cercariae to 50%, 12 mJ/cm² to 1%. >15 mJ/cm² reduced ability of cercariae to develop to worms to <1%. >60 mJ/cm² reduced motility immediately after irradiation. The difference between *S. mansoni* and *S. japonicum* was not significant.
Ariyo and Oyerinde, 1990	Effect of ultraviolet radiation on survival, infectivity and maturation of *Schistosoma mansoni* cercariae	Exposure also decreased fecundity of worms, shown by a reduction in egg load. Doses were not sufficient to prevent penetration. Used an activity score to characterise motility, activity significantly decreased in exposed cercariae four hours after exposure and mortality increased.
Kumagai *et al.*, 1992	Migration kinetics of ultraviolet-attenuated *Schistosoma mansoni* in ICR mice	Remaining water was checked after infection for non-penetrating cercariae but almost all were able to penetrate. Increase in fluence increases number of worms recovered in lungs and portal system, suggesting UV exposure prevents migration. At 15mJ/cm² no remaining worms were recovered.
Kamiya, 1993	Immunizing potential of ultraviolet-attenuated cercariae of *Schistosoma mansoni* in rodent hosts	Fluence did not affect motility or ability to penetrate, almost all cercariae were able to penetrate within 1 hour.
Mohamed, 1999	Scanning electron microscopical studies on the tegument of adult worms of *Schistosoma mansoni* originating from ultraviolet-irradiated and non-irradiated cercariae	Observation study on the structural changes to schistosomula and adult worms developed from irradiated cercariae. Significant damage to the tegument of male worms. Damage to female worms was less extensive. Some worms displayed sexual anomalies and were sterile.
Dajem and Mostafa, 2007	Scanning electron microscopical studies on *Schistosoma mansoni* cercariae exposed to ultraviolet irradiation	Observational study on the physical effects of UV on cercariae using SEM. UV did not appear to have any visible effects on the surface topography or cercariae. Suggests Schistosomes may have some ability to repair UV damage
Tian *et al.*, 2010	Immune Events Associated with High Level Protection against *Schistosoma japonicum* Infection in Pigs Immunized with UV-Attenuated Cercariae	Primarily about vaccine. No worms recovered from pigs exposed to UV attenuated cercariae, average of 328 worms recovered from pigs exposed to 1000 normal cercariae
Lin *et al.*, 2011	Multiple vaccinations with UV-attenuated cercariae in pig enhance protective immunity against *Schistosoma japonicum* infection as compared to single vaccination	Primarily about vaccine. Average of 31 worms recovered from pigs exposed to UV attenuated cercariae, average of 190 (approx. Taken from graph) worms recovered from pigs exposed to 1000 normal cercariae.
Konno, 1997	Effect of ultraviolet radiation of the infectivity of *Taenia taeniaeformis* eggs	Also carried out experiments on eggs with embryophore removed. Big variation in cyst development in control groups, unclear why. Very high fluence required to achieve significant log reduction, maybe because cyst development in control was also low.
Nolf, 1932	Experimental studies on certain factors influencing the development and viability of the ova of human *Trichuris* as compared with those of human *Ascaris* (Part III)	Trichuris was found to be significantly more resistant to UV than Ascaris. In other experiments higher inactivation's were achieved but the methods were less structured. Age/developmental stage of eggs is not specified.
Lipatov *et al.*, 2017	UV excilamp inactivation of helminth eggs in wastewater	Flow through reactor, unclear how fluence was determined. Number of eggs upstream of reactor not recorded and only 1 control and 2 experimental samples were taken - serious

		limitation to experiment. From previous study (unavailable) 15% were ale to survive fluence of 5.1mJ/cm^2 at 222nm and 70% were able to survive at fluence of 116mJ/cm^2 at 282 nm.
Nikulin, 1959	Destruction of *Ascaris lumbricoides* ova in pigsties by ultra-violet irradiation.	Repeatedly exposed to UV for 3-10 minutes in a number of sessions over a period of days. Some eggs were able to develop to intermediated stages but all were dead 50 days after irradiation.
Peng *et al.*, 2004	Effect of ultraviolet ray radiation on the development of *Ascaris lumbricoides* seminal eggs	Shows how UV damage manifests with time. After the first week of incubation development of irradiated eggs matched that of control group. After 2 weeks eggs exposed for 10-20 minutes began to show deformities. After 3 weeks control group had developed into larvae and were active, the 1 minutes group had developed but was inactive. Degeneration was apparent in some eggs exposed to higher fluences.
Stevens, 1909	The effect of ultra-violet light upon the developing eggs of *Ascaris megalocephala*	Exposure for 30 mins to 3 hours caused irregularities in development. Eggs were damaged but not necessarily killed after 8 hour exposure.
Shalimov, 1935	The influence of the ultra-violet light on the development of the eggs of parasitic worms: *Parascaris equorum* syn. *Ascaris megalocepahla*, *Enterobius vermicularis* and *Strongylus eqtnnus.*	UV caused irregular cell division, and unnatural development, eventually leading to death. Infective eggs/larvae can withstand substantially more UV, and required exposure for 25 minutes at least.
Williams and Colli, 1972	Influence of ionizing irradiation on infectivity of eggs of Echinococcus granulosus in laboratory rodents	No mice injected with 500 irradiated eggs developed infections, in comparison to 62% of mice injected with 500 control eggs. When inoculated 2000 irradiated eggs 75% mice developed infections, with 0.15% of eggs developing into cysts.
Ghandour and Webbe, 1974	Proceedings: The effect of ultra-violet irradiation on cercariae of *Schistosoma mansoni.*	Exposure for short periods had no visible effect on motility or behaviour however there was an 68% of cercariae died during skin penetration compared to 30% with control cercariae. No cercariae were able to develop into adult worms in the animal host, in contrast to 33% of control cercariae
Fang-Li *et al.*, 2010	Studies on resistance of mice vaccinated with uv-attenuated cercariae and schistosomula of *Schistosoma japonicum*	Primarily about vaccine. No worms recovered from mice exposed to 25-300 UV attenuated cercariae, average of 87 worms were recovered from mice exposed to 100 control cercariae.
Wang *et al.*, 1993	The effective period of immunization with UV-attenuated living cercariae vaccine against *S. japonicum* infection in mice	Primarily about vaccine. No worms recovered form mice exposed to 200 UV attenuated cercariae, average of 59 worms recovered from mice exposed to 200 control cercariae.
Zhang *et al.*, 2013	UV-attenuated *Schistosoma japonicum* cercariae induce immune responses in mice that ameliorate liver damage during subsequent infection with normal cercariae	Primarily about vaccine. There was no difference in mortality of cercariae (as tested with methylene blue stain) between irradiated and control cercariae.

Table A.3 List of papers excluded from the systematic review and code (duplicates not included to reduce size of table).

Code	Reference
2	Ravin, V. K.; Zamchuk, L. A. (1987). Action of UV Irradiation and Thymine Dimer Repair in the Nematode Caenorhabditis Elegans. *Radiobiologiia* 27 (3), 404–408.
2	Xia, F.; Lv, H.; Jiang, Y.; Tu, P. (2012). Simultaneous Determination of Benzophenones and Xanthone in Leaves of Aquilaria Sinensis by RP-HPLC-UV. *China Journal of Chinese Materia Medica* 40 (7), 1342–1346.
2	Mur, G. (1985). The control of in vitro development of parasitic fungi of the vine (*Phomopsis viticola, Uncinula necator*) by means of ultra-violet rays. *Progres Agricole et Viticole*
2	Boutry, J. L.; Barbier, M.; Ricard, M. (1976). The marine diatom *Chaetoceros simplex calcitrans* Paulsen and its environment. Effects of light and ultraviolet irradiation on the biosynthesis of fatty acids. *Comptes rendus hebdomadaires des seances de l'Academie des sciences. Serie D: Sciences naturelles* 282 (2), 239–242.
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2	Lv, L.; Diao, Q.; Zhao, Z.; Jia, L. (2017). Application of modified *Corylus heterophylla* fisch shell solid-phase extraction UV-Vis spectrophotometry for determination of malachite green in water. *Chemical Research and Application* 9.
2	Phương, N. T. K.; Dung, N. T.; Liên, N. K. (2007). Determination of presence of Arsenic (As) in plant (*Centella asiatica*) and broccoli (*Brassica juncea*) by atomic absorption spectroscopy (AAS) hydrogenation technique (HG). *Vietnam Journal of Chemistry* 45 (6), 691.
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A.2 Data analysis

Table A.4 Log reductions calculated from data extracted from sunlight, solar simulators, and long wavelength studies.

Species (Reference)	Lamp Type	Measure of inactivation	# organisms	Exposure Time (mins)	Log reduction	Interpolation Log reduction	Interpolation Exposure Time (mins)
A. caninum (Roh, 1968)	Sunlight	Visual	-	0	0.00	1	134
				60	0.09	2	180
				120	0.70		
				180	2.00		
A. suum, single cell (Spindler, 1940)	Sunlight	Larval development	5000	0	0.00	1	91
				30	0.01	2	125
				60	0.05	3	158
				180	3.68		
A. suum, embryonated (Spindler, 1940)	Sunlight	Larval development	5000	0	0.00	1	300
				120	0.08	2	311
				240	0.40	3	322
				300	0.98		
				330	3.68		
A. suum, single cell (Heaselgrave & Kilvington, 2011)	Solar simulator	Larval development	-	0	0.00	1	317
				60	0.01		
				120	0.08		
				240	0.24		
				360	1.42		
S. haematobium, miracidia (Prah and James, 1977)	Sunlight	Motility	-	30	0.00	1	196
				60	0.03		
				120	0.36		
				180	0.80		
				240	1.53		
A. ceylanium (Menon & Bhopale, 1985)	UV-A 390 nm	Worm burden	100	0	0.00	1	9
				1	0.17		
				15	1.61		
				30	1.61		
A. cantonensis (Kamath *et al.*, 1980)	UV-A 390 nm	Worm burden	100	0	0.00	1	4
				5	1.26		
				15	1.26		
S. mansoni, miracidia (Ruelas *et al.*, 2007)	UV-B 313 nm	Sporocyst development in snails	100	0	0.00	1	2.0
				0.43	0.00	2	2.5
				0.87	0.05		
				1.67	0.38		
				2.48	2.00		

Table A5. Log reductions calculated from data extracted from UV-C studies (fluence).

Species (Reference)	Lamp Type	Measure of inactivation	# organisms	Fluence (mJ/cm²)	Log reduction	Interpolation Log reduction	Interpolation Fluence (mJ/cm²)
Ascaris, embryonated (Mun, 2009)	LP	Larval development	300	0	0.00	1	3367
				2700	0.52	2	4748
				5400	2.47		
A. suum, single cell (Lucio-Forster *et al.*, 2005)	LP	Embryonation	-	0	0.00	1	84
				202	2.40	2	168
A. suum, single cell (Brownell & Nelson, 2005)	LP	Embryonation	300	0	0.00	1	100
				50	0.44	2	328
				100	1.00		
				200	1.56		
				300	1.91		
				400	2.23		
				500	2.53		
				800	2.53		
A. suum, decorticated single cell (Brownell & Nelson, 2005)	LP	Embryonation	-	0	0.00	1	30
				10	0.06	2	56
				20	0.52		
				30	1.01		
				40	1.53		
				50	1.80		
A. suum, embryonated (Tromba, 1978)	LP	Worm burden	-	0	0.00	1	11
				24	2.21	2	22
				48	1.86		
				93	2.29		
A. lumbricoides, single cell (de Lemos Chernicharo *et al.*, 2005)	LP	Embryonation	-	0	0.00	1	26
				6.7	0.51		
				13.6	0.73		
				20.3	0.77		
O. felineus, eggs (Lipatov et al., 2017)	Excimer	-	-	0	0.00	1	27
				25	0.92		
H. microstoma (Butler *et al.*, 1972)	-	Worm burden	10	0	0.00	1	619
				432	0.63		
				576	0.90		
				900	1.63		
S. japonicum (Lin *et al.*, 2011)	LP	Worm burden	1000	0	0.00	1	11
				24	2.28	2	21
S. japonicum (Shi *et al.*, (1990)	LP	Worm burden	1000	0	0.00	1	8
				24	3.04	2	16
S. japonicum Tian et al (2010)	LP	Worm burden	1000	0	0.00	1	5
				24	4.52	2	11
S. mansoni (Kumagai *et al.*, 1992)	LP	Worm burden	500	0	0.00	1	6
				2	0.03	2	9
				5	0.57		
				10	2.24		
				15	2.15		
S. mansoni (Kamiya, 1993)	LP	Worm burden	250	0	0.00	1	9
				18	1.91		
S. mansoni (Dean, 1983)	LP	Worm burden	500	0	0.00	1	7
				13.2	1.88		

				19.8	1.83		
S. mansoni and *S. japonicum* (Ruppel *et al.,* 1990)	LP	Motility	200 to 240	0	0.00	1	7
				3	0.30	2	12
				12	2.00		
S. japonicum (Fang-Li *et al.,* 2010)	-	Worm burden	25 to 300	0	0	1	9.60
				24	2.5	2	19.20
S. japonicum (Wang *et al.,* 1993)	-	Worm burden	200	0	0	1	13.56
				24	1.77	2	27.12
T. taeniaeformis, intact eggs (Konno, 1997)	LP	Cyst development	5000	0	0.00	1	872
				360	0.24	2	1300
				720	0.65		
				1440	2.33		
T. taeniaeformis, eggs with embryophore removed (Konno 1997)	LP	Cyst development	3000	0	0.00	1	10
				30	3.00	2	20
				60	3.00	3	30
				180	2.70		
				540	3.00		

Table A.6 Log reductions calculated from data extracted from UV-C studies (time).

Species (Reference)	Lamp Type	Measure of inactivation	# organisms	Exposure Time (mins)	Log reduction	Interpolation Log reduction	Interpolation Exposure Time (mins)
S. mansoni Standen and (Fuller, 1959)	LP	Motility	800	0	0.00	1	6.00
				2	0.30	2	8.10
				3	0.70		
				4	0.70		
				5	0.52		
				10	2.90		
S. mansoni (Ghandour and Webbe, 1975)	LP	Survival during skin penetration	1000-2000	0	0.00	1	0.96
				0.08	0.08	2	
				0.17	0.14		
				0.25	0.23		
				0.33	0.34		
S. mansoni (Ariyo and Oyerinde, 1990)	LP	Worm burden	50	0	0.00	1	0.46
				0.02	0.25	2	
				0.05	0.10		
				0.08	0.26		
				0.17	0.37		
H. diminuta – (McGanock & Howard, 1980)	LP	Cyst development	500-1000	0	0.00	1	16.00
				30	1.88		
F. gigantica (Riwidharso & Billalodin, 2006)	LP	Cercaraie development	-	0	0.00	1	1.69
				0.5	0.08		
				0.67	0.22		
				0.83	0.51		
				1.17	0.69		
A. caninum (Vierea & Rombert, 1974)	LP	-	-	0	0.00	1	11.78
				0.5	0.03		
				1	0.06		
				1.25	0.14		
				1.5	0.16		
				2	0.21		
				3	0.28		
				5	0.38		

Appendix B

B.1   Example ultraviolet light-emitting diode fluence calculation

```matlab
%% UV LED FLUENCE DETERMINATION %%
Exp_Date = datetime([2020 12 8]);

%Import LED Spectral Power Distribution
SPD = importdata('LED_SPD.csv');
%Import radiometer sensor callibration data (e.g. SED 270) for your device
C = importdata('Callibration_Dec2019.csv');

%LED nominal wavelength (e.g. 255, 265, or 285 nm)
LED = 285;
%Volume of solution added to Petri dish (mL)
v_sol = 3.8;
%Volume of stir bar (mL)
v_bar = 0.024;
%Petri dish internal diameter (cm)
dia = 2.89;
%Distance from LED to Petri dish surface (cm)
L = 10.4;
%Absorbance of sample at LED weighted average wavelength (cm^-1)
alpha = 0.007;
%Grid size - e.g. for a measurement grid from -1.5 to 1.5 enter 1.5
G = 1.5;

%Irradiance BEFORE experiments (mW/cm^2)
E_before = [0.634 0.663 0.679 0.685 0.677 0.662 0.630
            0.666 0.691 0.707 0.712 0.707 0.688 0.661
            0.682 0.710 0.724 0.728 0.724 0.707 0.676
            0.690 0.711 0.728 0.734 0.730 0.715 0.686
            0.681 0.708 0.724 0.730 0.724 0.705 0.670
            0.663 0.695 0.709 0.715 0.708 0.688 0.654
            0.635 0.664 0.680 0.687 0.680 0.660 0.630];

%Required fluences
F_req = [0 50 100 150 200 300];

%Irradiance AFTER experiments (mW/cm^2) - If you haven't carried out the
%experiment yet, enter 0
E_after =  [0.630 0.669 0.683 0.685 0.678 0.661 0.631
            0.666 0.690 0.707 0.714 0.707 0.689 0.660
            0.681 0.709 0.723 0.729 0.724 0.704 0.671
            0.690 0.713 0.729 0.734 0.728 0.710 0.678
            0.678 0.706 0.724 0.729 0.722 0.705 0.674
            0.662 0.692 0.708 0.712 0.705 0.688 0.653
            0.629 0.664 0.678 0.681 0.675 0.655 0.625];
```

```matlab
%%%%%%%%%%%%%%%%DO NOT MAKE ANY CHANGES BELOW THIS LINE%%%%%%%%%%%%%%%%

fprintf('\nDate: %s \nNominal Wavelength: %3.0f nm\n',Exp_Date,LED)

v = v_sol+v_bar; %Total volume in Petri dish (mL)
A = pi*(dia/2)^2; %Petri dish area (cm)
l = v/A; %Water path length (cm)
F_req = F_req.'; %Required fluences

%%% CORRECTION FACTORS %%%

RF = 0.975; %Reflection factor
WF = (1-10^(-alpha*l))/(alpha*l*log(10)); %Water factor
DF = L/(L+l); %Divergence factor
S_lamda = C(:,2)/max(C(:,2)); %Sensitivity of radiometer relative to peak sensitivity
if LED == 255
    E_lamda = SPD(:,2)/sum(SPD(:,2)); %Select relvant SPD and normalise
elseif LED == 265
    E_lamda = SPD(:,3)/sum(SPD(:,3));
elseif LED == 285
    E_lamda = SPD(:,4)/sum(SPD(:,4));
end
SF = 1/sum(S_lamda.*E_lamda); %Sensor Factor

factors = [RF, WF, DF, SF];
fprintf('_____\n \nCorrection Factors:\n')
fprintf('RF %5.4f\nWF %5.4f\nDF %5.4f\nSF %5.4f\n',factors)

%%% AVERAGE IRRADIANCE ON SAMPLE SURFACE (BEFORE) %%%

%Create polar grid
[x,y] = meshgrid(-G:0.5:G); %Measurment grid dimensions (Cartesean) (cm)
rs = 0.1; %Radius spacing of polar grid (cm)
ths = 2*pi/360; %Theta spacing of polar grid (radians)
r = [rs/2:rs:(dia/2-rs) dia/2]; %Define polar grid locations
th = (ths/2:ths:(2*pi-ths/2));
[R,TH] = meshgrid(r,th); %Create grid
[xq,yq] = pol2cart(TH,R); %Calculate cartesian coordinates for polar grid locations

%Interpolate irradiance measurements (before) to polar grid and multiple by SF
E_rth_before = interp2(x,y,E_before,xq,yq)*SF;

%Area of each polar grid location A(r,th) (cm)
A_rth = zeros(size(E_rth_before));
for i = 1:length(th)
    for j = 1:length(r)
        if r(j) == rs/2
            A_rth(i,j) = rs^2*ths/2; %Area of first sector, closest to centre
        elseif ((rs/2) < r(j)) && (r(j) < (dia/2))
            A_rth(i,j) = ((r(j)+(rs/2))^2-(r(j)-(rs/2))^2)*ths/2;
        else
            A_rth(i,j) = ((dia/2)^2-(r(j-1)+(rs/2))^2)*ths/2; %Area of last sector,
                closest to perimeter
```

```matlab
        end
    end
end
%Weighted area of each polar grid location (no units)
A_weighted = A_rth/A;

%Average irradiance on sample surface (before) (mW/cm^2)
E0bar_before = sum(sum(A_weighted.*E_rth_before))/sum(sum(A_weighted));

%%% CV CHECK (BEFORE) %%%
%Coeffieceint of variation
CV_before = sqrt(sum(sum(A_weighted.*((E_rth_before-E0bar_before).^2)))/sum(sum(
    A_weighted)))/E0bar_before;
CV_before = CV_before*100;

%%% AVERAGE FLUENCE RATE IN SAMPLE (BEFORE) AND EXPOSURE TIMES TO ACHIEVE REQUIRED
    FLUENCES %%%

if CV_before >= 6.7
    fprintf('CV (before) > 6.7%% - check PearlBeam set up')
else
    fprintf('CV (before) %2.1f %%\n',CV_before)
    Ebar_before = (E0bar_before*RF*WF*DF); %Average fluence rate in sample (mW/cm^2)
    t_1=1/Ebar_before; %Exposure time for 1 mJ/cm^2
    t_sec = t_1*F_req; %Exposure time in seconds for required fluences
    t = datestr(seconds(t_sec),'HH:MM:SS'); %Convert exposure times to MM:SS

    %Display required exposure times to command window
    fprintf('_____\n \nRequired Exposure Time (MM:SS):\n')
    disp(t)

%%% AVERAGE IRRADIANCE ON SAMPLE SURFACE (AFTER) %%%

    if E_after == 0
        disp('On completion of experiments please enter "Irradiance AFTER experiments
            (mW/cm^2)" and re-run code to calculate the average fluences')
    else
        %Interpolate irradiance measurements (after) to polar grid and multiple by SF
        Ep_after = interp2(x,y,E_after,xq,yq)*SF;

        %Average irradiance on sample surface (after) (mW/cm^2)
        E0bar_after = sum(sum(A_weighted.*Ep_after))/sum(sum(A_weighted));

%%% CV CHECK (After) %%%

        %Coeffieceint of variation
        CV_after = sqrt(sum(sum(A_weighted.*((Ep_after-E0bar_after).^2)))/sum(sum(
            A_weighted)))/E0bar_after;
        CV_after = CV_after*100;

%%% AVERAGE FLUENCE RATE IN SAMPLE (AFTER) AND ACTUAL APPLIED FLUENCES %%%

        if CV_after >= 6.7
```

```matlab
            fprintf('CV (after) > 6.7%% — check PearlBeam set up')
        else
            fprintf('_____\n \nCV (after) %2.1f %%\n',CV_after)
            Ebar_after = (E0bar_after*RF*WF*DF); %Average fluence rate in sample (mW/
                cm^2)
            Ebar = mean([Ebar_before, Ebar_after]); %Average of the fluence rate (
                before) and (after) calculations
            fprintf('_____\n \nAverage Applied Fluence Rate (mW/cm^2):\n')
            fprintf('%5.4f\n',Ebar)
            F_Ave = Ebar*(t_sec); %Applied fluence based on exposure times (mJ/cm^2)
            fprintf('_____\n \nAverage Applied Fluence (mJ/cm^2):\n')
            fprintf('%3.0f\n',F_Ave)
```

```
>> Fluence_Determination_20201208_285
    Date: 08—Dec—2020
    Nominal Wavelength: 285 nm
    _____
    Correction Factors:
    RF 0.9750
    WF 0.9953
    DF 0.9469
    SF 1.0681
    CV (before) 2.1%
    _____
    Required Exposure Time (MM:SS):
    00:00:00
    00:01:11
    00:02:23
    00:03:35
    00:04:47
    00:07:10
    _____
    CV (after) 2.1%
    _____
    Average Applied Fluence Rate (mW/cm^2):
    0.6956
    _____
    Average Applied Fluence (mJ/cm^2):
    0
    50
    100
    150
    200
    300
>>
```

B.2 Example low pressure mercury arc lamp fluence calculation

Germicidal Fluence (UV Dose) Calculations for a Low Pressure UV Lamp
Programmed by Jim Bolton - Bolton Photosciences Inc., 628 Cheriton Cres., NW, Edmonton, AB, Canada T6R 2M5
Tel: 780-439-4709; Fax: 780-439-7792; Email: jbolton@boltonuv.com
Comments and/or questions are welcome

DO NOT CHANGE ANY CELLS OTHER THAN THE CELLS WITH A YELLOW BACKGROUND

INSTRUCTIONS AND NOTES

1. Set up a 'quasi' collimated beam apparatus. A 'collimating tube' may be used, but it is preferable to use circular 'masks' to define the beam. Make sure that safety measures are taken to protect workers from exposure to the UV from the lamp. EYE PROTECTION IS AN **ABSOLUTE** REQUIREMENT.
2. Place the detector head of the UV radiometer on a horizontal surface, containing a 0.5 cm x 0.5 cm grid, such that the 'calibration plane' (see the Calibration Sheet provided by the manufacturer of the Radiometer) is at the level of where the top of the solution will be during exposures to the UV.
3. Determine the Petri Factor using the procedure given starting in Column **Q**.
4. Make sure that the spectrophotometer is balanced with distilled water in the same cuvette. Then measure the absorption coefficient (1 cm absorbance) at 254 nm for the water to be irradiated and insert into Cell **G37**.
5. Insert the solution volume added to the Petri dish into Cell **G31**.
6. Insert the stirrer volume into Cell **G32**.
7. Insert the internal Petri dish diameter into Cell **G33**.
8. Insert the distance from the center of the UV lamp to the surface of the water in the Petri Dish into cell **G36**.
9. Insert the center meter reading into cell **G40**.
10. Insert the desired Fluences (UV Doses) into cells **E46 to E52**.
11. Remove the radiometer detector head and place a Petri Dish (or other container), containing the cell suspension, on a stirring motor placed so that the top of the solution is at the same level as that of the calibration plane of the detector head. Add a very small stir bar and make sure that the stirring rate is such that there is no vortex.
12. Expose samples in the UV beam for the times calculated in rows **46 to 52**.
13. For a control, or zero dose determination, a sample solution is added to the Petri dish, set into the collimated beam apparatus and left stirring for a time equal to the average of the exposure times used. The resulting counts are used as the 0 dose concentration

solution volume added to Petri dish =	3.8 mL	Water Factor =	0.9874
stirrer volume =	0.024 mL	Divergence Factor =	0.9809
Petri dish internal diameter =	2.89 cm	Reflection Factor =	0.9750
total volume in Petri dish =	3.824 mL	Petri Factor =	0.9729
water path length =	0.58 cm		
distance from UV lamp to top of water surface =	30 cm		
absorption coefficient at 254 nm =	0.0190 cm^{-1}		
total absorbance at 254 nm =	0.0111		

Radiometer reading at the center of Petri Dish =	0.248 mW/cm^2	
True irradiance across the Petri dish =	0.241 mW/cm^2	

Average Germicidal Irradiance throughout the water volume =   0.228 mW/cm^2

						min	seconds
Time for a Fluence (UV Dose) of	1	mJ/cm^2 =	4.389 s				
Time for a Fluence (UV Dose) of	50	mJ/cm^2 =	219.4 s	=		3	39
Time for a Fluence (UV Dose) of	100	mJ/cm^2 =	438.9 s	=		7	19
Time for a Fluence (UV Dose) of	150	mJ/cm^2 =	658.3 s	=		10	58
Time for a Fluence (UV Dose) of	200	mJ/cm^2 =	877.8 s	=		14	38
Time for a Fluence (UV Dose) of	300	mJ/cm^2 =	1316.7 s	=		21	57
Time for a Fluence (UV Dose) of		mJ/cm^2 =	0.0 s	=		0	0
Time for a Fluence (UV Dose) of		mJ/cm^2 =	0.0 s	=		0	0

Determination of the "Petri Factor"

Steps:
1. Draw a 0.5 cm x 0.5 cm grid and place the center of the grid at the center of the collimated beam
2. Measure the UV irradiance (with a radiometer) every 0.5 cm in the *x* and *y* directions and place the readings into columns **S** and **X** below. It is only necessary to take readings out to 0.5 cm beyond the radius of the Petri dish (or other container to be used). The units do not matter since ratios are calculated.
3. The Petri factor is calculated in Cell **CW151** and is also displayed in Cell **K34**.

UV Distribution across the Petri Dish for a low pressure UV Lamp

x	y	Meter Reading	Ratio	x	y	Meter Reading	Ratio
0	-2.0	0	0.000	-2.0	0	0	0.000
0	-1.5	244	0.984	-1.5	0	231	0.931
0	-1.0	245	0.988	-1.0	0	236	0.952
0	-0.5	246	0.992	-0.5	0	242	0.976
0	0.0	248	1.000	0.0	0	248	1.000
0	0.5	247	0.996	0.5	0	245	0.988
0	1.0	247	0.996	1.0	0	242	0.976
0	1.5	247	0.996	1.5	0	239	0.964
0	2.0	0	0.000	2.0	0	0	0.000

B.3 Actinometry protocol and example calculation

Based on methods by Bolton *et al.* (2011) and Kheyrandish *et al.* (2018), with minor adjustments.

1. Prepare 100 mL of KI/KIO_3 actinometry stock solution in a volumetric flask as follows (use as soon as possible and always within 4 hours):

   - 9.96 g potassium iodide (KI)
   - 2.14 g potassium iodate (KIO_3)
   - 0.381 g Sodium tetraborate decahydrate ($Na_2B_4O_7 \cdot 10H_2O$)
   - 100 mL RO water

2. Measure the absorbance of 3.8 mL of stock solution using a quartz cuvette (10 mm path length) at 300 nm – this should be 0.58.

3. Measure the irradiance with the radiometer at the centre of the exposure area.

4. Adjust bench jack so that the top of the Petri dish is at the same level as the calibration plane of the radiometer detector.

5. Add a magnetic stir bar and 6.6 mL of stock solution to a 30 mm Petri dish.

6. Place on stirrer in the centre of the exposure area. Stir for 30 seconds. Leave for 3 minutes, without opening shutter. Measure the absorbance of 3.8 mL of the blank solution at 352 nm – this should be 0.02.

7. Repeat step 5. Place on stirrer in the centre of the exposure area. Stir for 30 seconds. Open shutter and expose to UV for 3 minutes. Close shutter once timer sounds. Measure the absorbance of 3.8 mL of the sample at 352 nm. Repeat at least two more times.

8. Repeat steps 5 and 6.

9. Return bench jack to correct height for radiometer detector. Measure the irradiance with the radiometer at the centre of the exposure area.

10. Measure the absorbance of 3.8 mL of the stock solution at 300 nm.

Table B.1 List of reagents.

Reagent	Provider	Catelogue No.
Potassium iodide	Fischer Chemical	10703811
Potassium iodate	Fischer Chemical	10746301
Sodium tetraborate decahydrate	Sigma	S9640

```matlab
%% UV LED ACTINOMETRY CALCULATION %%
Exp_Date = datetime([2021 06 03]);

%Import LED Spectral Power Distribution for your device
SPD = importdata('LED_SPD.csv');
%Import radiometer sensor callibration data (e.g. SED 270) for your device
C = importdata('Callibration_Dec2020.csv');

%LED nominal wavelength (e.g. 255, 265, or 285 nm)
LED = 265;
%Distance to surface of radiometer and sample surface (cm)
L = 10.4;
```

```matlab
%Volume of solution added to Petri dish (mL)
v_sol = 6.6;
%Petri dish internal diameter (cm)
dia = 2.89;
%Actinometry exposure time (s)
t_act = 180;
%Grid size - e.g. for a measurement grid from -1.5cm to 1.5cm enter 1.5
G = 1.5;

%Irradiance BEFORE actionmetry exposures (mW/cm^2)
E_before = [0.179 0.187 0.191 0.193 0.190 0.185 0.177
            0.187 0.195 0.200 0.202 0.199 0.194 0.185
            0.193 0.201 0.206 0.207 0.205 0.200 0.193
            0.196 0.203 0.208 0.209 0.208 0.203 0.194
            0.195 0.203 0.207 0.208 0.206 0.201 0.193
            0.190 0.198 0.203 0.204 0.202 0.197 0.187
            0.181 0.189 0.194 0.196 0.194 0.189 0.180];

%Absorbance at 352nm of blank solution (cm^-1)
alpha_352b = [0.038 0.029];

%Absorbance at 352nm of exposed solution (cm^-1)
alpha_352e = [1.283 1.262 1.239];

%Irradiance AFTER actionmetry exposures (mW/cm^2)
E_after =  [0.179 0.187 0.191 0.193 0.190 0.185 0.177
            0.187 0.195 0.200 0.202 0.199 0.194 0.185
            0.193 0.201 0.206 0.207 0.205 0.200 0.193
            0.196 0.203 0.208 0.209 0.208 0.203 0.194
            0.195 0.203 0.207 0.208 0.206 0.201 0.193
            0.190 0.198 0.203 0.204 0.202 0.197 0.187
            0.181 0.189 0.194 0.196 0.194 0.189 0.180];

%%%%%%%%%%%%%%%%%DO NOT MAKE ANY CHANGES BELOW THIS LINE%%%%%%%%%%%%%%%%%

fprintf('\nDate: %s \nNominal Wavelength: %3.0f nm \nDistance to Surface: %3.1f cm\n '
    ,Exp_Date,LED,L)

A = pi*(dia/2)^2; %Petri dish area (cm^2)
delta_alpha = mean(alpha_352e)-mean(alpha_352b); %Difference in absorbance at 352nm (
    cm^-1)
c = 2.9979E8; %Speed of light (m.sec^-1)
h = 6.63E-34; %Plancks constant (J.sec)
N = 6.022E23; %Avagadro's constant (mol^-1)
epsilon = 27636; %Molar absorbtion coefficient at 352nm (M^-1.cm^-1)

%%% ACTINOMETRY FLUENCE RATE CALCULATION %%%

QY = importdata('QY_Literature.csv'); %Import Quantum Yield Literature Data
SPD_QY = SPD;
SPD_QY([1:2,61:76],:) = []; %Reduce LED SPD to wavelength range with QY data (205-309
    nm)
```

```matlab
E_ave = (E_before+E_after)/2; %Mean measured irradiance (before and after exposures)
if LED == 255 %Select relevant SPD (units arbitary)
    E_lamda_QY = SPD_QY(:,2);
elseif LED == 265
    E_lamda_QY = SPD_QY(:,3);
elseif LED == 285
    E_lamda_QY = SPD_QY(:,4);
end

%Interpolate spectral distribution of QY
lamda_Lit = QY(:,1); %Wavelengths in literature (nm)
phi_Lit = QY(:,9); %Avereage QY at given wavelengths in literature
lamda = (205:2:319)'; %Required wavelengths (nm)
phi_lamda = interp1(lamda_Lit,phi_Lit,lamda); %Interpolate QY for full range (205-309
    nm)

%Calculate spectral distribution of photon energy
lamda_m = lamda/1E9; %Convert wavelength from nm to m
U_lamda = c*h*N./lamda_m; %Energy of a photon at wavelength lamda (J)

%Fluence rate on surface (mW/cm^2)
E_0 = (delta_alpha*v_sol/(epsilon*t_act*A))*trapz(lamda,U_lamda.*E_lamda_QY./phi_lamda
    )/trapz(lamda,E_lamda_QY);

%%% RADIOMETER IRRADIANCE %%%

%Sensor Factor
S_lamda = C(1:76,2)/max(C(1:76,2)); %Sensitivity of radiometer relative to peak
    sensitivity
if LED == 255
    E_lamda = SPD(:,2)/sum(SPD(:,2)); %Select relvant SPD and normalise
elseif LED == 265
    E_lamda = SPD(:,3)/sum(SPD(:,3));
elseif LED == 285
    E_lamda = SPD(:,4)/sum(SPD(:,4));
end
SF = 1/sum(S_lamda.*E_lamda);

%Create polar grid
[x,y] = meshgrid(-G:0.5:G); %Measurment grid dimensions (Cartesean) (cm)
rs = 0.1; %Radius spacing of polar grid (cm)
ths = 2*pi/360; %Theta spacing of polar grid  (radians)
r = [rs/2:rs:1.45 1.455]; %Define polar grid locations
th = (ths/2:ths:(2*pi-ths/2));
[R,TH] = meshgrid(r,th); %Create grid
[xq,yq] = pol2cart(TH,R); %Calculate cartesian coordinates for polar grid locations

%Interpolate average irradiance measurements to polar grid nd mulitply by SF
E_rth = interp2(x,y,E_ave,xq,yq)*SF;

%Area of each polar grid location A(r,th) (cm)
A_rth = zeros(size(E_rth));
for i = 1:length(th)
```

```matlab
    for j = 1:length(r)
        if r(j) == rs/2
            A_rth(i,j) = rs^2*ths/2; %Area of first sector, closest to centre
        elseif ((rs/2) < r(j)) && (r(j) < (dia/2))
            A_rth(i,j) = ((r(j)+(rs/2))^2-(r(j)-(rs/2))^2)*ths/2;
        else
            A_rth(i,j) = ((dia/2)^2-(r(j-1)+(rs/2))^2)*ths/2; %Area of last sector,
                closest to perimeter
        end
    end
end
%Weighted area of each polar grid location (no units)
A_weighted = A_rth/A;

%Average irradiance on surface (mW/cm^2)
E0bar = sum(sum(A_weighted.*E_rth))/sum(sum(A_weighted));

%%% CV CHECK %%%
%Coeffieceint of variation
CV = 100*sqrt(sum(sum(A_weighted.*((E_rth-E0bar).^2)))/sum(sum(A_weighted)))/E0bar;

if CV >= 6.7
    disp('CV > 6.7% - check PearlBeam set up')
else
    %Percentage Difference
    PD = 100*(E_0-E0bar)/(0.5*(E_0+E0bar));
    fprintf('_____\n \nFluence Rate (Radiometry) %5.4f mW/cm^2 \nIrradiance (
        Actinometry) %5.4f mW/cm^2 \nPercentage Difference %5.4f\n\n',E0bar,E_0,PD)
end

>> Actinometry_June2021_265

    Date: 03-Jun-2021
    Nominal Wavelength: 265 nm
    Distance to Surface: 10.4 cm

    _____

    Fluence Rate (Radiometry) 0.2253 mW/cm^2
    Irradiance (Actinometry) 0.2362 mW/cm^2
    Percentage Difference 4.7348

>>
```

B.4 Linear regression data and statistical tests

Figure B.1 Ultraviolet fluence-response of *Schistosoma mansoni* cercariae 0 minutes post-exposure. Red symbols indicate data points that were not included in linear regression because they were outside the linear range or because all cercariae in the sample were dead. Equation represents log reduction (y) as a function of fluence (x).
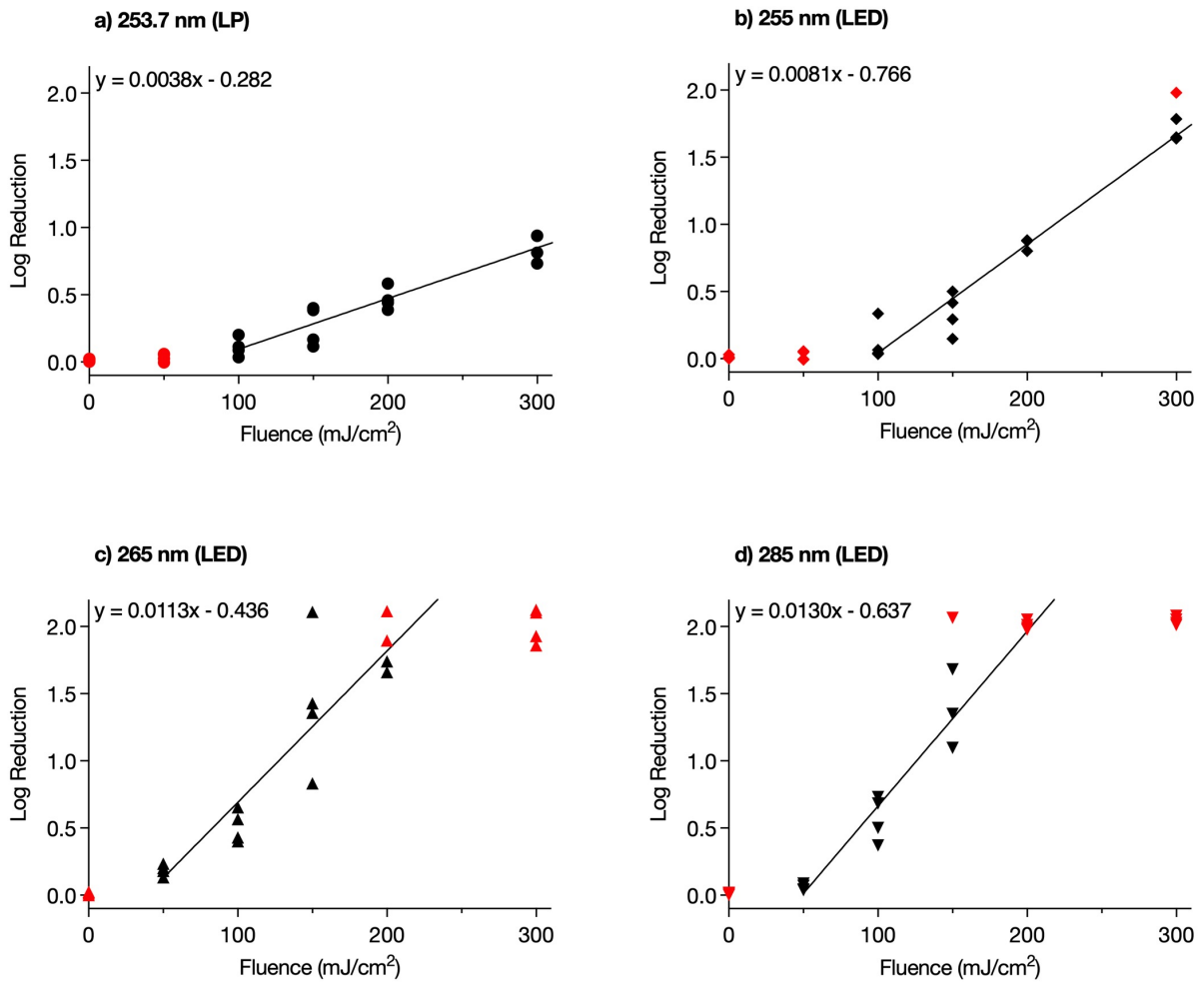
Figure B.2 Ultraviolet fluence-response of *Schistosoma mansoni* cercariae 60 minutes post-exposure. Red symbols indicate data points that were not included in linear regression because they were outside the linear range or because all cercariae in the sample were dead. Equation represents log reduction (y) as a function of fluence (x).
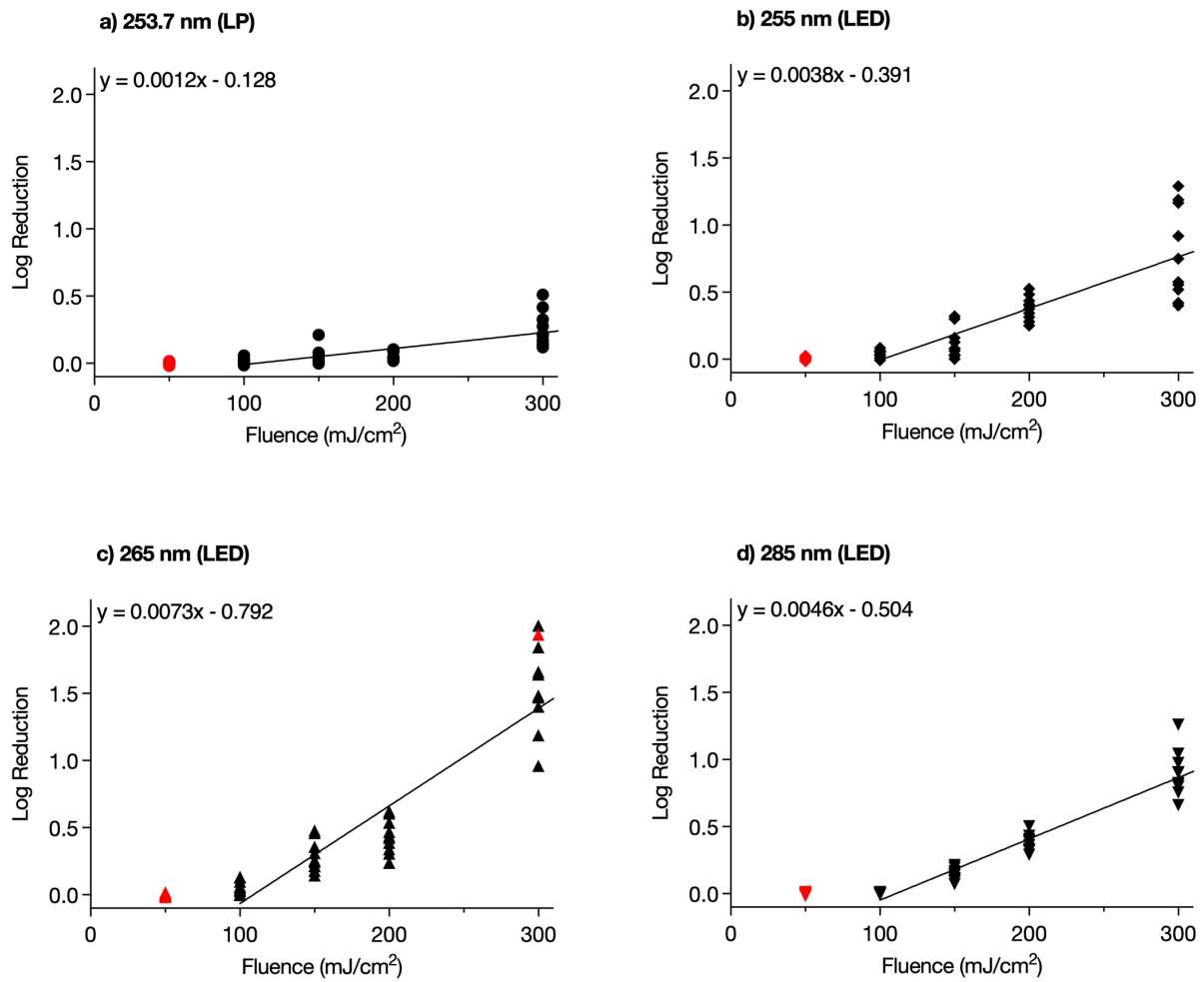
Figure B.3 Ultraviolet fluence-response of *Schistosoma mansoni* cercariae 180 minutes post-exposure. Red symbols indicate data points that were not included in linear regression because they were outside the linear range or because all cercariae in the sample were dead. Equation represents log reduction (y) as a function of fluence (x).
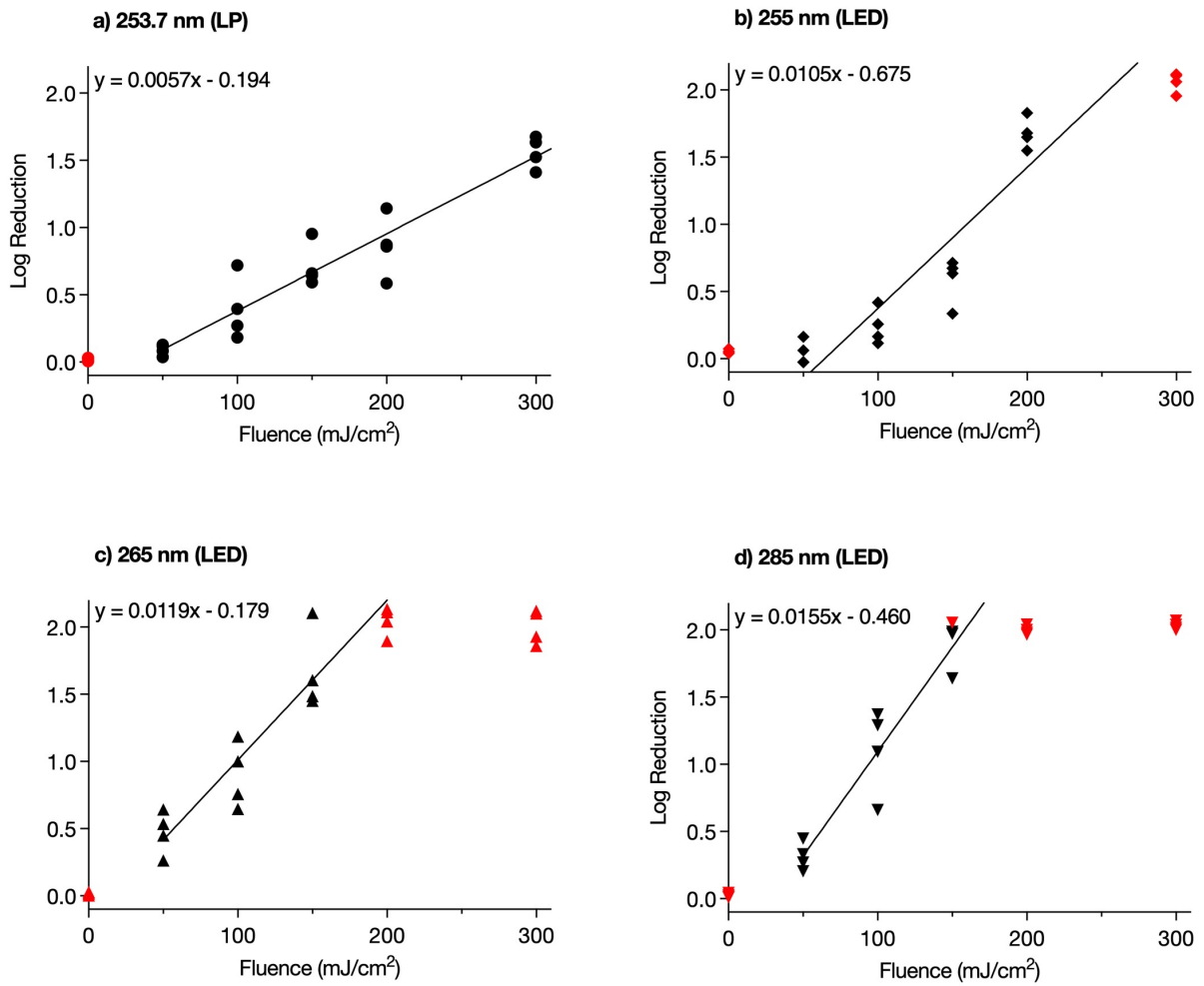
Table B.2 Inactivation rate constants were compared using a one-way analysis of variance followed by Tukey's multiple comparisons test. If the slopes of two lines were statistically similar (p >0.05), an extra-sum-of-squares F-test was carried out to determine if the two data sets could be fit to a shared model, to confirm similarity.

Tukey's multiple comparisons test of inactivation rate constants (k_D)		
0 mins	**Adjusted P Value**	**Significance**
LP vs. 255 LED	<0.0001	****
LP vs. 265 LED	<0.0001	****
LP vs. 285 LED	<0.0001	****
255 LED vs. 265 LED	<0.0001	****
255 LED vs. 285 LED	0.4500	ns
265 LED vs. 285 LED	<0.0001	****
60 mins	**Adjusted P Value**	**Significance**
LP vs. 255 LED	0.0145	*
LP vs. 265 LED	<0.0001	****
LP vs. 285 LED	<0.0001	****
255 LED vs. 265 LED	0.1277	ns
255 LED vs. 285 LED	0.0119	*
265 LED vs. 285 LED	0.6786	ns
180 mins	**Adjusted P Value**	**Significance**
LP vs. 255 LED	0.0128	*
LP vs. 265 LED	0.0024	**
LP vs. 285 LED	<0.0001	****
255 LED vs. 265 LED	0.8473	ns
255 LED vs. 285 LED	0.0309	*
265 LED vs. 285 LED	0.2309	ns
Extra sum-of-square F-test of slope (k_D) and y-intercept (c)		
0 mins	**P Value**	**Significance**
255 LED vs. 285 LED	0.2316	ns
60 mins	**P Value**	**Significance**
255 LED vs. 265 LED	<0.0001	****
265 LED vs. 285 LED	0.7587	ns
180 mins	**P Value**	**Significance**
255 LED vs. 265 LED	<0.0001	****
265 LED vs. 285 LED	0.2598	ns

Appendix C

C.1 Deoxyribonucleic acid concentrations

Table C.1 DNA concentration of each test sample, each sample was assayed in duplicate.

Fluence (mJ/cm^2)	DNA Concentration (ng/mL)		
	255 nm	265 nm	285 nm
0	12.10	19.30	8.84
0	14.00	14.70	14.30
10	-	11.10	15.00
10	-	16.00	12.60
25	9.30	19.80	11.40
25	10.70	18.80	11.10
50	9.11	14.50	15.60
50	11.60	15.20	14.40
100	10.00	19.60	11.10
100	9.53	14.80	12.90
150	-	19.70	10.60
150	-	17.20	15.90

C.2 List of reagents and kits.

Table C.2 List of reagents and kits.

Reagent/Kit	Provider	Catelogue No.
DNeasy Blood & Tissue Kit	Qiagen	69504
Qubit dsDNA HS Assay Kit	ThermoFischer	Q32851
OxiSelect UV-Induced DNA Damage (CPD Quantitation) ELISA kit	Cell Biolabs	STA-322
Tris EDTA Buffer	Sigma-Aldrich	PPB010
Phosphate buffered saline tablets	Sigma-Aldrich	P4417-50TAB
Fluoresceine diacetate	Sigma-Aldrich	F7378-5G
Propidium iodide solution	Sigma-Aldrich	P4864-10ML

C.3 Preliminary fluorescence assay results
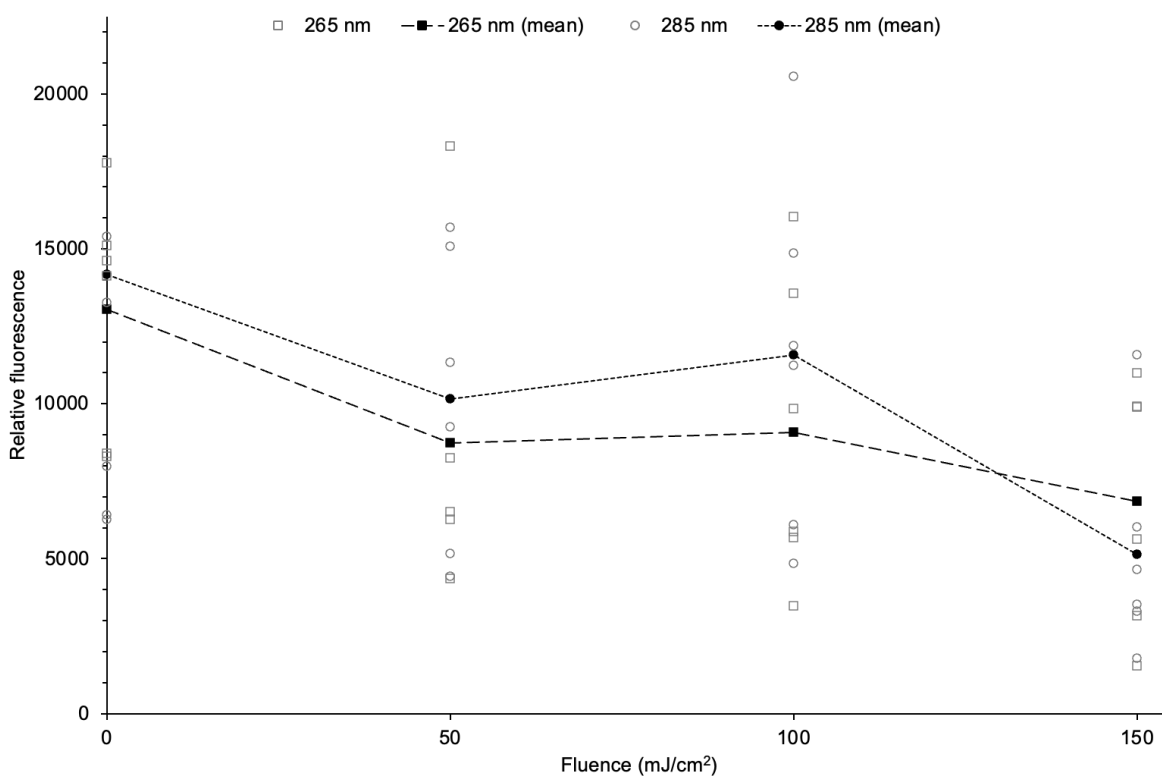
Figure C.1 Preliminary fluoresceine diacetate fluorescence data. fluoresceine diacetate is a vital dye that is only retained in live cells. Variations in the number of cercariae per well lead to high variation in the results.
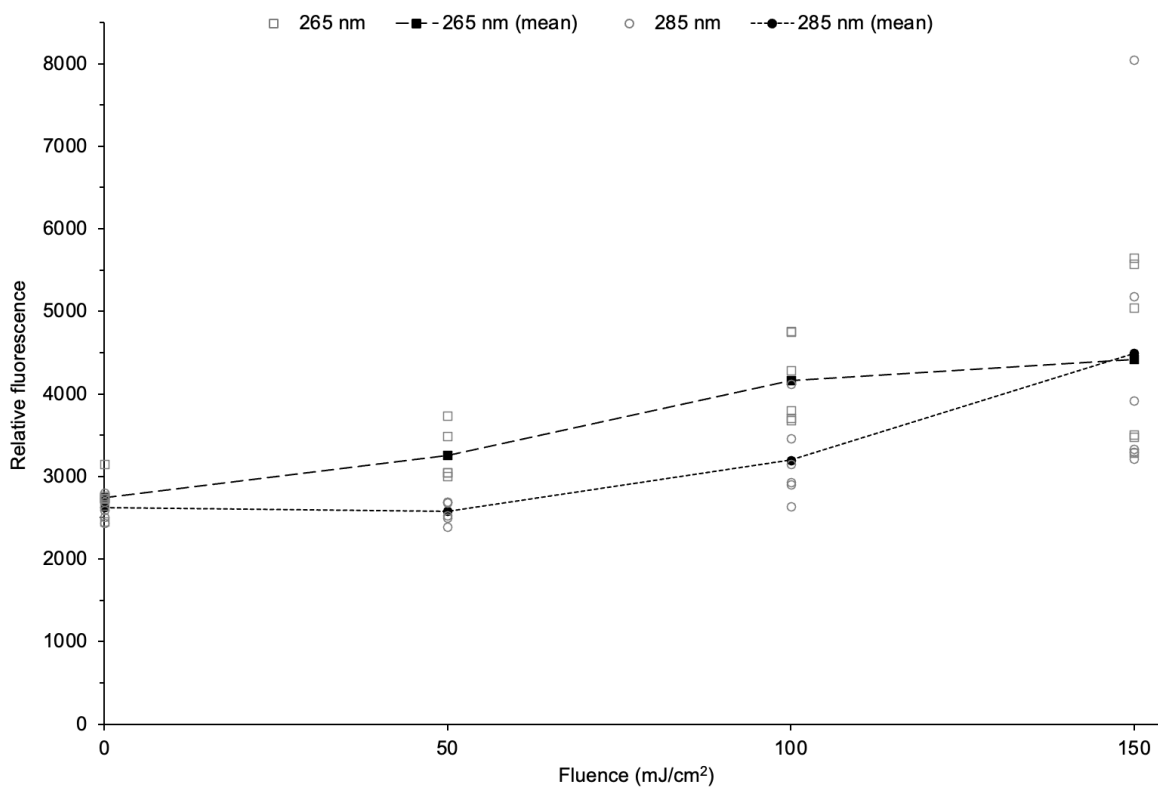
Figure C.2 Preliminary propidium iodide fluorescence data. PI is a non-vital dye that is only able to stain deoxyribonucleuc acid if the cell membrane has been breached.

Appendix D

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