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Reverse osmosis and ultrafiltration for recovery and reuse of larval rearing water in *Anopheles arabiensis* mass production: Effect of water quality on larval development and fitness of emerging adults

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ABSTRACT

Background: Countries around the world are showing increased interest in applying the sterile insect technique against mosquito disease vectors. Many countries in which mosquitoes are endemic, and so where vector control using the sterile insect technique may be considered, are located in arid zones where water provision can be costly or unreliable. Water reuse provides an alternate form of water supply. In order to reduce the cost of mass rearing of *Anopheles arabiensis* mosquitoes, the possibility of recycling and reusing larval rearing water was explored.
Methods: The used rearing water (‘dirty water’) was collected after the tilting of rearing trays for collection of larvae/pupae, and larvae/pupae separation events and underwent treatment processes consisting of ultrafiltration and reverse osmosis. First-instar *An. arabiensis* larvae were randomly assigned to different water-type treatments, 500 larvae per laboratory rearing tray: ‘clean’ dechlorinated water, routinely used in rearing; dirty water; and ‘recycled’ dirty water treated using reverse osmosis and ultrafiltration. Several parameters of insect quality were then compared: larval development, pupation rate, adult emergence, body size and longevity. Water quality of the samples was analyzed in terms of ammonia, nitrite, nitrate, sulphate, dissolved oxygen, chloride, and phosphate concentrations after the larvae had all pupated or died. Surface water temperatures were also recorded continuously during larval development.
Results: Pupation rates and adult emergence were similar in all water treatments. Adult body sizes of larvae reared in recycled water were similar to those reared in clean water, but larger than those reared in the dirty larval water treatment, whereas the adult longevity of larvae reared in recycled water was significantly increased relative to both ‘clean’ and ‘dirty’ water. Dirty larval water contained significantly higher concentrations of ammonium, sulfate, phosphate and chloride and lower levels of dissolved oxygen than clean water. These parameters significantly varied during the period of larval development. After dirty water was recycled by ultrafiltration and reverse osmosis, all the parameters measured were the same as those in clean water.

Abbreviations: SIT, sterile insect technique; RH, relative humidity; IPCL, insect pest control laboratory; FAO, food and agricultural organization; IAEA, international atomic energy agency; LD, light: dark; UF, ultrafiltration; RO, reverse osmosis; DO, dissolved oxygen.

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Conclusion: This study demonstrated the potential for using recycled larval rearing water to supplement clean dechlorinated water supplies. Recycling used water improved its quality and of the reared mosquitoes. As water demands and environmental pressures grow, recycling of larval rearing water will improve the sustainability and affordability of mosquito mass-rearing.

1. Introduction

Mosquitoes are among the most notorious vectors of human diseases and destroy more lives on a yearly basis than any forms of violence and other human diseases combined (Leal, 2014). Although a huge international effort has cut malaria mortality rates by about half since 2000, it was still responsible for 438,000 deaths worldwide in 2015 (WHO, 2015). In regions where malaria is endemic such as Sub-Saharan Africa, malaria morbidity and mortality continues to result in human and economic disability. In the absence of effective vaccines, vector control is the primary means to prevent disease and this has relied mainly on the use of various insecticides. However, mosquito resistance to chemical insecticides (Hemingway, 2014; Raghavendra et al., 2011) is a growing problem, leading to increased attention being paid to alternative control methods. There has been a considerable increase in interest in applying the sterile insect technique (SIT) against mosquitoes in the wake of the global Zika endemic, and it may be effective against malaria vectors. The SIT is based on the release of large numbers of reproductively sterile insects into a wild population of the same species, with the result that sterile males mate with wild females and impede their reproduction (Knipling, 1955).

The success of mosquito mass-rearing for the sterile insect technique (SIT) or other mass-release-based applications relies on a reliable supply of water of sufficient quality, as all mosquito immature stages need water to develop prior to becoming adults. More than a billion people currently live in water-scarce regions, and as many as 3.5 billion could experience water scarcity by 2025. As the world's population continues to grow, pressure on water supplies will continue to increase. Moreover, global warming arising from human activity (greenhouse gas emissions) is further increasing the probability of conditions leading to prolonged periods of drought. Many countries in which mosquitoes are endemic are located in arid zones where water provision can be costly or unreliable. A mass-rearing system needs to be cost effective, and water saving methods may be an important part of achieving this in a sustainable manner in some regions. Water is the medium through which larvae receive all essential macro- and micronutrients and oxygen. If clean water is used for all these purposes without considering recovery and recycling possibilities, a huge amount of water would be required. For example, around 250 l of water are required per FAO/IAEA larval mass-rearing rack (which can hold up to 200,000 *Anopheles* larvae, or about 1 million *Aedes* larvae) making the availability of clean water and disposal of wastewater key considerations in the running of a mosquito mass production facility. Recycling is the treatment process which enables the used larval rearing water to be purified to the extent that will allow it to be reused to rear successive generations of larvae.

The Insect Pest Control Laboratory (IPCL) of the joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has initiated activities to explore the possibility of reusing larval water to rear successive batches of mosquito larvae (Mamai et al., 2016). This use is expanding in order to accommodate the needs of the environment and growing water supply demands. There are multiple technologies that can be used to treat water that may include irradiation such as UV (Backer, 2002), ultrafiltration (UF) (Bohdziewicz et al., 2003; Boudaud et al., 2012), or ultrasonic treatments (Bazyar et al., 2013), heat treatment, or autoclaving. Water treatment employing UF and reverse osmosis (RO) membrane processes are frequently implemented for the production of high-quality recycled water. UF and RO are pressure driven membrane operations. The process is driven by a pressure gradient that forces water molecules across a semi-permeable membrane. Any material larger than the pores in the membrane will be removed on a size exclusion basis (i.e. by sieving). UF is required for the removal of colloids, suspended and macromolecular matter and virus, while RO is even suitable for the removal of bacteria, viruses, dissolved species, mineral substances and low-molecular organic compounds (Bohdziewicz et al., 2003; Jadhao and Dawande, 2012; Wintgens et al., 2005). When rearing in the FAO/IAEA larval mass-rearing rack, water can be collected when the rack is tilted to collect pupae and used to rear the next round of mosquitoes. First results appeared promising, with *Anopheles arabiensis* larvae developing well to adulthood (Mamai et al., 2016) when reared in previously used rearing water. However, larval water cannot simply be reused for production of *An. arabiensis* larvae without investigating its impact on the quality of adult insects. Therefore, the specific questions being addressed in this study are: i) does recycled dirty larval water affect the quality of mosquitoes produced? ii) can water treatment processes be used to render the water reusable without any detrimental effects on the mosquitoes produced? This second question can be answered by: i) determining the chemical properties of the dirty larval water and variation over the course of larval development and ii) investigating the influence of recycling dirty larval water (using UF and RO treatments) on the water's chemical properties and development and quality of *An. arabiensis*.

2. Methods

2.1. Mosquito colony and rearing conditions

Experiments were performed using an *An. arabiensis* Dongola strain originating from the Northern State of Sudan. The colony has been maintained at the IPCL since 2005 under controlled conditions (27 ± 1 °C, 70% ± 10% relative humidity (RH), 12:12 h light:dark (LD), including one hour dusk and one hour dawn).

Mosquitoes were reared following the *An. arabiensis* mass-rearing protocols described in Balestrino et al. (2012), Maiga et al. (2016) and Mamai et al. (2016). Fresh batches of *An. arabiensis* eggs collected from mass-rearing cages (Mamai et al., 2017) were hatched and reared to pupation in the larval mass-rearing rack (Balestrino et al., 2012, 2014). Each tray was filled with 4 l of deionized water the day before adding the eggs to allow the water to reach room temperature. Using the egg quantification method described in Maiga et al. (2016), an aliquot of 4000 eggs was added to each of the 50 trays in a plastic ring floating on the surface of the water. Larvae were fed with the FAO/IAEA diet suspension (5 g/L tuna meal, 5 g/L bovine liver powder, and 4.6 g/L vitamin mix:) following the published protocol (IAEA, 2015), and 24 h after the first pupae were observed the rack was tilted to collect the larvae and pupae. The resulting 'dirty' water was passed through a 50-μm sieve (Retsch® Test Sieve with steel mesh) to remove all eggs,