**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.

**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.

**Key words:** Sterile insect technique, Mass production, *Anopheles arabiensis,* Water recycling, Ultrafiltration, Reverse osmosis, Chemical properties.

**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.

**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](http://www.un.org/waterforlifedecade/scarcity.shtml) currently live in water-scarce regions, and [as many as 3.5 billion](http://siteresources.worldbank.org/IDA/Resources/IDA-Water-Resources.pdf) 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 liters 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 re-used 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 hr 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), Maigaet 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 de-ionized 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 4,000 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 hours 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, remaining larvae and debris and the water was retained for use in the following experiments.

Approximately 90% of the initial 200 L was recovered.

*2.2. Dirty water treatment*

Fifty litres of the dirty larval water collected as described in the previous section was recycled using a water treatment apparatus (SFW machine: dimensions 138 × 48 × 48 cm, Switzerland) developed by the Swiss Fresh Water SA (SFW) company (SFW). The system is based on UF and RO, which remove particles larger than 0.0001 microns (µm) and 99% of salts. The cycle begins with a pre-filter, followed by UF, where bacteria, viruses and parasites are removed. Afterwards, it enters an RO membrane where mercury, arsenic, fluoride and salt are eliminated and the “recycled” water extracted for use. The machine used in this study produces up to 170 litres of premium quality water per hour (4,000 to 14,000 L of potable water per day), and is adapted to the harshest conditions, using solar energy (SFW).

*2.3. Effect of recycled larval rearing water on quality of mosquitoes*

The first-instar *An. arabiensis* larvae that were used in this experiment were obtained by hatching fresh batches of eggs collected from mass-rearing cages in standard laboratory rearing trays (40×30×7 cm). Trays were assigned randomly to different water-type treatments. Each tray was filled with 1 L of one of three water treatments: clean water, dirty water and recycled water. Each treatment tray was replicated three times. Five hundred larvae were added to each tray, and reared until pupation, following the protocol previously described by Mamai et al., (2016). Briefly, a 1% suspension of the FAO/IAEA larval food was added daily to each larval tray in the following amounts: 10 mL for 1st and 2nd day, 20 mg for 3rd and 4th day and 30ml for the remaining days until pupation.

Pupae were removed on a daily basis, counted and placed into small vessels containing 50 ml of the same water treatment as they had been reared as larvae. These vessels were put in individual cages (30×30×30 cm, BugDorm-1H; MegaView, Taichung, Taiwan) until the adults emerged. The rate of pupation for each water type was calculated as the total number of pupae obtained at the end of the development period over the initial number of L1s. Dead pupae were counted to calculate the rate of emergence as a proportion of adults emerging from the total number of pupae.

After emergence, 50 males and 50 females from each cage and each water type were transferred and maintained in a cage together (15×15×15 cm, Bugdorm.com, Taiwan) for measurement of longevity. A 5% sugar solution was supplied in a 150-ml plastic bottle containing a filter paper and mortality was determined daily.

To determine whether larval-rearing water affected adult body size, the right wings of 45 to 50 females and males per treatment (about 15 wings per replicate) were detached and mounted on glass microscope slides under a cover slip. A photograph of each wing was taken under a dissecting microscope (Leica MZ16 FA, Leica Microsystems (Switzerland) Ltd). Wing length was measured from the tip of the wing (excluding fringe) to the distal end of the alula (Lyimo and Koella, 1992) using analySIS® FIVE software. Wing length is considered to be a proxy for mosquito body size (Fernandes and Briegel, 2005).

*2.4. Water sample collection and chemical analyses*

During the experiment described above, water was sampled daily from each tray, starting from the day before adding the L1 larvae (day zero) until day 6 when pupation started. Approximately 15 ml of water was collected from each tray and stored in sterile conical tubes at -20°C until further processing.

Water temperature and dissolved oxygen (DO) were monitored *in situ* using a calibrated Dissolved Oxygen/°C Meter (OAKTON® Mettler, DO 100 series, serial number 94449, Singapore). The water samples were analyzed for inorganic chemical parameters at the Austrian Institute of Technology (AIT) laboratories. Ammonium (NH4) and nitrite (NO2) were measured using standard colorimetric-based biochemical analysis (Hood-Nowotny et al., 2010). Standards were prepared fresh for ammonium, from an NH4Cl stock solution (1000 mg N/l, stable at 4°C for several weeks) in the respective extractant by serial dilution ranging from 20 to 0.312 mg/l. For nitrite, standards were prepared using 4 ppm N as NO3- in the range of 4 to 0.062 ppm by serial 1:2 dilution. Concentrations were obtained from the corresponding standard curve prepared by linear regression. Nitrate (NO3), bromide (Br), fluoride (F), chloride (Cl), phosphate (PO4) and sulfate (SO4) concentrations were determined by high-performance anion-exchange chromatography (HPAEC) (DIONEX MODEL DX-120 Ion Chromatograph) (Hood-Nowotny et al., 2010).

*2.5. Statistical analysis*

Statistical analyses were performed using SPSS V13 (IBM, USA) and GraphPad Prism 5.0 softwares. Pupation and emergence data, expressed as a percentage, were arcsine transformed to stabilize variance and normalize distribution. Pupation, adult emergence and wing length variables were compared between treatments using a one-way ANOVA followed when required by a Tukey’s *post-hoc* test. Adult longevity was estimated using the Kaplan-Meier method. The results from different water-type treatments were compared using the log-rank test. Inorganic chemical parameters were analyzed using General Linear Model (GLM) multivariate analysis. We used a post hoc test (Tukey’s *post-hoc* test) to determine the difference in each chemical concentration between the different water types.

**3. Results**

*3.1. Effect of recycled larval water on quality of mosquitoes*

Total pupation ranged from 85.74±0.36 to 93.87±2.04% of initial L1s and was unaffected by rearing water type (Table 1, ANOVA, F=2.985, df=2, *P*=0.1610). The emergence rate varied from 96.36±2.59 to 99.52±0.47% of initial pupae, and did not differ significantly between water treatments (Table 1, ANOVA, F=1.432, df=2, *P*=0.3223). The average time to pupation ranged from 7.92±0.16 to 8.23±0.44 days, and no difference was found in time to pupation between treatments (Table1, ANOVA, F= 0.2611, df= 2, *P*= 0.7801).

Body size was affected by water treatments in both males and females (Figure 1, ANOVA, F=46.99, df=5, *P*<0.0001). Dirty water resulted in smaller mean body size compared to clean water (Tukey's *post-hoc* test *P*>0.05). Using recycled water resulted in significantly increased wing length in both males and females compared to dirty water (Tukey’s *post-hoc* test, *P*<0.05).

Adult longevity was significantly affected by water treatment (graphical observation, Figure 2, Log-rank (Mantel-Cox) test, χ*²*=129.0, df=5, *P*<0.0001); summarized in Table 2. Recycled larval water significantly increased the longevity of adult females compared to both dirty (Gehan-Breslow-Wilcoxon test, χ²=29.21, df=1, *P*<0.0001) and clean water (χ²=87.40, df=1, *P*<0.0001 and Gehan-Breslow-Wilcoxon test χ²=11.19, df=1, *P*=0.0008). The same effect of the recycled water was observed on male longevity (Gehan-Breslow-Wilcoxon test, χ²=72.69, df=1, *P*<0.0001) and (χ²=14.12, df=1, *P*=0.0002) compared to dirty and clean water, respectively. Whatever the water type treatment, males survived longer than females and this is likely due to absence of females blood feeding and not the norm.

*3.2. Physico-chemical parameters of larval rearing water*

The mean temporal variation in chemical parameters of different water types is presented in Table 3. A significant larval-water type × time of collection interaction was observed for all measured parameters except for fluoride (Table 4). There were several statistically significant differences in mean measurements of water quality between the three larval water types (Table 4); ammonium, chloride, phosphate and sulfate concentrations were significantly higher in the dirty water (Table 3). No significant differences were found in these parameters between clean and recycled water (*P*>0.05), however the concentration of dissolved oxygen in dirty water was significantly lower (range of 1.77 mg/l to 4.62 mg/l) with a trend of increasing with time, whereas the opposite trend was measured in clean and recycled waters. Most of the physico-chemical parameters showed significant variation over time (Table 3). Significant concentrations of neither nitrite nor nitrate were detected in all larval water types, though minimal concentrations of nitrite were detected in the last days of the dirty water treatment.

**4. Discussion**

For both as SIT component and other vector control strategies relying on large-scale production of mosquitoes which includes an aquatic larval phase, the availability of sufficient water of a constant quality is an essential requirement. *An. arabiensis* is considered a primary vector in arid environments, being found in dry environments, savannah and sparse woodlands (Gillies and de-Meillon, 1968; Sinkaet al., 2010). The increasing demand for access to clean water, especially in regions that have an arid or semi-arid climate and where SIT could be implemented, necessitates water reuse to supplement the finite water supply. Around 100,000 l of water is required to produce 10,000,000 sterile males per week (Mamai, pers. comm.). Water quality is an important determinant of whether or not eggs will hatch and the resulting immature stages will successfully complete their development to the adult stage (Piyaratneet al., 2005). A recent study demonstrated that *An. arabiensis* can be reared in water previously used to rear a cohort of the same species without any effect on hatch rate, larval development time, or mortality in the aquatic stages of development. However, a major concern in reusing larval-rearing water is the carry-over effects on adult fitness including adult body size and survival, which may potentially influence the success of the SIT component. Therefore, treating the water in some way before it is re-used is a good solution.

Recycled water has been used for a number of purposes such as agricultural and landscape irrigation, industrial processes, sanitation, replenishing a ground water basin or even for drinking (Anderson, 2003). In this study, combined use of UF and RO proved to be effective in creating a suitable water supply for *An. arabiensis* rearing, at least for one subsequent cohort of mosquitoes. Although this method has reached its aim, this is not a single specific technology for water treatment and processing high volumes of water during the overall mass-rearing process may represent a significant expense. This cost of water treatment technologies certainly adds to the operation costs within mass-rearing facility. Emergency plans, such as backup water systems, may be needed as measures that can contribute to increasing coping capacity in case of water shortage, as in areas with recurrent droughts. However, if implemented early enough in an SIT, this environmental-friendly approach would be sustainable and cost-effective in the long term, compared to water supply alternatives such as use of potable water or imported water, particularly if recycling of water proves to be possible for multiple consecutive rearing cohorts. It confers safety insofar as the rearing facility would no longer rely on tap water in countries where quality problems and supply interruptions frequently happen. It confers also ecological and economic advantages in that it reduces the volume of water required without adding significant costs to the rearing process.

In these experiments, dirty water was found to have increased levels of ammonium, sulfate, phosphate and chloride, chemicals among the indicators of water pollution (Florescuet al., 2011; Kabulaet al., 2011). Nitrogenous wastes are poisonous to aquatic organisms at certain concentrations; for example, ammonium can have a toxic effect, especially on fish, above 0.2 mg/l (Florescuet al., 2011). Various chemical properties of the larval habitat, including pH, temperature, and concentration of ammonia, nitrate and sulphate have been found to affect mosquito larval development and survival (Briegel, 2003; Muteroet al., 2004). Thus, the negative impact of reused larval-rearing water on mosquito body size and longevity was not surprising. Several studies have indicated that the physio-chemical characteristics of larval habitats were found to be key factors determining the presence, survival and population dynamics of immature *Anopheles* (Muturi et al., 2008; Mwangangia et al., 2007; Rejmankova et al., 1991). Moreover, Diabate et al., (2005) suggested that *An. coluzzii* and *An. gambiae* successfully exploit different habitats with regard to the chemical, physical, and microbiological conditions. Although *An. arabiensis* developed well to adulthood in dirty water (Mamaiet al*.*, 2016), Ye-Ebiyo et al., (2003) found that excessive turbidity impeded the production of numerous and large adult *An. arabiensis* in the laboratory setting. He explained it by the fact that the presence of inert particles (waste materials such as dead and decomposing larvae, discarded exoskeletons, excretory products and surplus food) suspended in the larval environment may prevent mosquito larvae from feeding effectively. Dirty larval water could possibly contain high concentrations of inert particles that could affect the ability of these mosquito larvae to extract nutrients.

Dissolved oxygen concentration was lower in dirty water and consistently less than 50% saturation (5mg L-1). Oxygen is essential for all organisms including insects, and generally, an increase in dissolved oxygen is beneficial to aquatic ecosystems (Moniz, 2013). It has been shown that moderate chronic hypoxia has significantly reduces survival rates in fish embryos (Bardon-Albaret and Saillant, 2016), and *Anopheles* larvae similarly depend mainly on cutaneous respiration and their surface area is limited; although they have breathing tubes they are rudimentary. Many authors have demonstrated that the presence and abundance of *Anopheles* larvae were positively associated with concentration of dissolved oxygen in oviposition sites (Dejenie *et al.*, 2011; Muturi et al., 2008), for example Oyewole et al., (2009). This data suggests that *Anopheles* really are adapted to oligotrophic low nutrient waters with concomitant higher oxygen tension and suffer both in terms of adult survival and growth when oxygen levels are low. Indeed the significantly higher oxygen concentrations observed in the recycled water could be the key factor determining increased adult survival compared to the clean water treatment. This theory would have to be tested in more depth as correlation does not necessarily prove causality and there may be another explanation. If proven, though, adding simple bubbling water pumps to larval rearing trays could increase productivity and male survival after release and thus increase operational efficiency.

In contrast to dissolved oxygen, ammonium, sulfate, phosphate and chloride were found in lower concentrations or were absent in recycled water, certainly as a result of the treatment process. Furthermore treating water before reusing significantly improved the longevity of *An. arabiensis* adults compared to those reared not only to dirty water, but particularly in the clean water routinely used for rearing. Additionally, we found no decrease in adult body size relative to clean water though this was seen with the dirty larval water. For sterile males release program to be successful, they must disperse into the environment, survive long enough to locate and attract wild females, and copulate competitively with wild females (Perez-Staples and Shelly, 2013). Although the effect of each chemical on body size and survival was not assayed in this study independent of other changes in the water, they may have been at least a contributing factor in reducing the quality of adults. Although it has been previously shown that higher dietary nitrogen levels can lead to larger adult mosquitos (Hood-Nowotnyet al., 2012), nitrogen pollution in the form of highly toxic non-ionised ammonia (NH3) or relatively non-toxic ammonium (NH4+) could also have played a role in the lower survival and body weight of the adults reared in dirty water. The pH of all water was below 7 (tested using universal indicator strips at day 6), which suggests that overall nitrogen content of the water would have been in the form of ammonium based on equilibrium kinetics (Nayloret al., 2014). Toxic effects of the nitrite in the later stages of larval development may have led to the depression of growth and survival. However it has been shown that nitrite concentrations greater than 1 ppm are suboptimal for the growth of prawn larvae (Gomeset al., 2016), and since we observed concentrations of around 10 ppb hypoxia rather than nitrite toxicity was likely to be the cause of the suboptimal survival and growth. It was interesting, however, to see these levels of nitrite rise as the denitrification bacterial population came to dominate in the dirty water; zero nitrate values (measured but not shown) suggest that all the observed ammonium losses form the dirty water system were attributable to denitrification. It is possible that other variables which were not measured, such as the presence of bacteria or other pathogens, may have contributed to the reduced adult quality. It would be interesting in further studies to characterize the changing bacterial communities over the course of larval development, or at least to compare the composition before and after the water was used for rearing *Anopheles*, in comparison to water treated similarly but in the absence of mosquito larvae.

**5. Conclusion**

The effects of using recycled larval rearing water on mosquito rearing and the quality of resulting adults was assessed in this study. Dirty larval water was characterized by high concentrations of ammonium, sulfate, phosphate and lower DO concentrations. This dirty water negatively affected adult body size and longevity. The recycling process may have significantly improved water quality by its effects on inorganic components, increasing the longevity of the resulting adults. This experience has demonstrated that recycled rearing water is a valuable resource in rearing *An. arabiensis* mosquitoes and can effectively replace the clean dechlorinated water routinely used, if the right treatment regime is in place.

**Competing interests**

The authors have declared that they have no competing interests.

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**Authors’ contributions**

WM, RH performed the experiments. WM drafted the manuscript which was critically revised by RH, YH, RSL and JRLG. HM, SNBS, ABA, DDS and HY participated in experiments and data analysis. WM, RH, RSL and JRLG conceived the study and JRLG supervised the entire work. All authors read and approved the final version of the manuscript.

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**Table 1** Mean ((±se) time to pupation, pupation and emergence rates of *Anopheles arabiensis* reared in different larval water treatments.

|  |  |  |  |
| --- | --- | --- | --- |
| **Water type** | **Time to pupation (days)** | **Pupation rate (%)** | **Emergence rate (%)** |
| **Clean water** | 8.14±0.26 | 85.74±0.37 | 99.52±0.48 |
| **Dirty water** | 7.92±0.16 | 88.25±2.53 | 96.36±2.59 |
| **Recycled water** | 8.23±0.44 | 93.86±2.04 | 99.32±0.19 |

**Table 2** Mean (±se) longevity (in days) of *Anopheles arabiensis* male and female adults reared in different larval water treatments (clean, dirty and recycled waters; n= 3 replicates, 50 mosquitoes/replicate)

|  |  |  |  |
| --- | --- | --- | --- |
| **Sex** | **Clean water** | **Dirty water** | **Recycled water** |
| **Male** | 16.95 ± 0.63 | 12.65 ±0.60  | 20.69 ±0.53  |
| **Female** | 10.42 ±0.40  | 8.84 ±0.35  | 12.53 ±0.45  |

**Table 3** Temporal variation and mean (±se) of the physical and chemical characteristics of different larval rearing waters during the rearing of larvae in the different water treatments. For each parameter, different superscripts indicate means are significantly different (GLM, Tukey’s *post-hoc test, P<0.05*).

**Table 4** Results of multivariate analysis (GLM) for the effects of larval water type, day of collection and their interaction on the chemical parameters.

**Figure legends**

**Figure 1** Mean wing-length in male and female *Anopheles arabiensis* adults reared from L1 in different larval water treatments.Different letters indicate significantly different results between treatments, by sex (ANOVA, *P<0.05*).

**Figure 2** Longevity of male (A) and female (B) *Anopheles arabiensis* adults reared from L1 in clean, dirty and recycled waters.