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Changes in Water Quality Threaten the Spread of Infectious Diseases; Filaria Vector *Culex pipiens* Dealing with Water Contaminants

Mahmoud M. Zidan[1]*, Samia E. El-Didamony[2], Fatma Z. Hamed[2], Ahmed S. Hussein[1]

[1]Zoology Department, Faculty of Science (Boys), Al-Azhar University, Cairo - 11884, Egypt
[2]Zoology Department, Faculty of Science (Girls), Al-Azhar University, Cairo - 11884, Egypt

***Corresponding Author: Mahmoud.zidan@azhar.edu.eg**

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ABSTRACT

In Egypt, the most common mosquito-borne diseases transmitted to human beings are exacerbated by water contamination caused by the unregulated discharge of chemical contaminants. Population growth and urbanization have expanded possible habitats, resulting in an increase in the abundance of *Culex pipiens*. In the present study, the fifth generation was reared at 27± 2°C, 75% RH, and a photoperiod of 12:12h (light-dark) in clean, moderately, and contaminated water to determine the impact of water contaminants on the population dynamics of *Culex pipiens*. Our results showed that *C. pipiens* had a finite growth rate (λ) of 1.3116 d-1, an intrinsic growth rate (r) of 0.3145 d-1, a net reproductive rate (R0) of 100.06 offspring, and a mean fertility (F) of 262.53 eggs in highly contaminated water. These values are much higher than those found in the offspring of water with minimal contamination. While there was no significant difference in male adult lifetime across treatments, female adult lifespan was significantly reduced in clean water (9.91 days) and moderately polluted (10.54 days) water compared to contaminated water (13.63 days). In moderately and clean water, the parameters were as follows: λ = 1. 2311 d-1, 198.02 eggs, r = 0.2105 d-1 and R0 = 68.45 offspring, λ = 1.169 d-1, 186.92 eggs, r = 0. 2047 d-1, and R0 = 64.09 offspring, respectively. This research suggests that *Culex pipiens* may undergo the degree of adaptation to severely contaminated water, which might do enough to increase the diversity of the mosquito-borne disease.

INTRODUCTION

Mosquitoes are a biological vector of several diseases such as filaria, malaria, and dengue and the Rift Valley fever. In Egypt, the most common mosquito-borne diseases transmitted to human beings are: the West Nile virus **(Abdel-Hamid *et al.*, 2011a, b)**, malaria and the dengue fever **(Abdel-Hamid *et al.*, 2013)**. There is a wide diversity of aquatic environments where eggs are deposited. Most species of *Culex* lay their eggs in water collected on the ground, such as in pools, puddles, ditches, borrow pits and rice field. Certain eggs are incubated in tin cans, water containers, bottles, and water storage tanks **(Service, 2012)**. The World Health Organization (WHO) predicted in 2020 that there will be 241 million cases of malaria worldwide, with 627,000 fatalities, a number only marginally lower than that recorded in 2010 and 2017 **(WHO, 2020, 2021)**.

Egypt has the continent’s second-highest population, behind only Nigeria. The Nile Valley and Delta, which make up just 4% of Egypt’s total territory, are where the vast majority of the country’s urbanization has taken place due to the country’s unique geography and climate **(UNODC, 2005)**. For decades, it was Egypt’s principal malaria vector, and it sometimes causes malaria in the Sinai Peninsula's southern and southeasters regions, even today **(Lotfy, 2014)**. Adult mosquito populations are expected to have an effect on the spread of lymphatic filariasis and malaria due to the prevalence of *Anopheles* and *Culex* larvae **(Castro *et al.*, 2010)**. Lymphatic filariasis, malaria and schistosomiasis and the Rift Valley fever are only few of the at least vector-born illnesses probably avoided by taking precautions that have been shown efficient **(WHO, 2019, 2020)**. One other measure of water quality is the amount of dissolved organic matter (DOM) found in water **(Williams *et al.*, 2016; Tang, 2019)**. DOM is the most abundant form of organic matter in aquatic systems, and nutrient contamination may significantly alter its concentration and composition **(Xenopoulos *et al.*, 2021)**.

Aquatic physicochemical variables may have a role in malaria outbreaks since they affect *Culex* survival, development, and reproduction. Except for those four communities, where *An. maculipennis* and *C. torrentium* were predominated, *C. pipiens* was the most common species of mosquito. Electrical conductivity, alkalinity, total hardness, and chloride were significantly correlated positively with the density of *C. pipiens*, but no significant negative correlations were found **(Dida *et al.*, 2018; Abdel-Meguid, 2022)**. Although *Culex* mosquitoes prefer clean water for breeding, it is possible for their larvae to mature in polluted water **(Ye, 2011; Emidi *et al.*, 2017)**. Aquatic environments are now more polluted as a result of human residential via the discharge of wastewater, the disposal of solid waste, and the use of fertilizers, insecticides, and detergents **(Chen *et al.*, 2015; WHO, 2023)**. Water contamination might become worse in the future due to climate change and other environmental factors **(Matus *et al.*, 2008; Shellenberger & Nordhaus, 2009**; **Manciocco, 2014)**. It is possible that mosquitoes may develop resistance to these chemicals **(Goselle *et al.*, 2018)**. Therefore, it is crucial to learn how water pollutants affect *Culex* population health **(Ukubuiwe *et al.*, 2020)**. Disease epidemiology relies heavily on environmental factors, such as the survival, development, and fertility of vector populations at different life stage **(Yurttas & Alten, 2006)**.

This research aimed to investigate the adaptation potential of *C. pipiens* at breeding sites with varied degrees of pollution by comparing the demographic parameters of *C. pipiens* in clean, moderately and severely polluted water.

MATERIALS AND METHODS

Culex pipiens colony

In this section, the protocols followed by the Medical Entomology Department's animal house at Al-Azhar University, where the colony has survived for at least five generations will be discussed in detail. Each tray of *C. pipiens* eggs (24 hours) was placed in one liter and half of tap water **(Meuti *et al.*, 2023)**. The trays were 30 centimeters in length, 20 centimeters in breadth and 15 centimeters in height. An environment of 27± 2°C, 75± 5% RH and a photoperiod of 12:12h (light-dark) was maintained for the trays. Larvae were fed a piece of bread every day for the first 24 hours after hatching **(Loetti *et al.*, 2011)**. When the fourth instars appeared, the trays were daily examined to see whether any pupae had emerged. The recovered pupae were put in plastic cups with tap water. A total of 30 pairs of male and female adults were placed in a screen-rearing cage (35 x 35 x 40cm) to breed.

Adults were provided with a sugar solution (10% sugar) soaked in cotton wicks contained in a vial. To avoid the formation of mold and germs, every two days, the cotton wicks and sugar solution were changed. Every three days, all mosquito females were fed on bleed for 90 minutes. Mosquito eggs were collected by placing a white enamel dish (180ml tap water capacity, 8cm diameter, 20cm height) in the cage. The dish was checked daily, and any freshly laid eggs were transferred to trays filled with tap water.

Preparation of polluted water and pollutants

Phosphate, nitrite, nitrate, and total organic carbon (TOC) used in this research are the common pollutants in aquatic environment. Water samples having three different qualities were prepared: clean water treatment (i.e., control treatment), moderately polluted water, and highly polluted water. Deionized water (DI) was used to make the stock solutions and contaminated water. Clean water was prepared with tap water and deionized water (1:1 in ratio). All tap waters used for preparation of treatment waters were set aside for 24h in the laboratory to remove chlorine **(Fazeli-Dinan *et al.*, 2022)**.

Data in Fig. (1) provide information on the levels of phosphate, nitrite, nitrate, and total organic carbon (TOC), present in lab-created clean, moderately and extremely contaminated water samples (TOC). Highly contaminated water has the highest allowable levels of all components, according to the World Health Organization **(Frisbie *et al.*, 2012)**. To prepare water samples for the analysis of phosphate, nitrite, nitrate, and total organic carbon (TOC) concentrations, sodium nitrate ($NaNO_3$), chloramine (NH_2CL), humic acid, and chlorine (Cl_2) were added to clean water **(Zazouli *et al.*, 2007)**. Total organic carbon (TOC), a surrogate for the presence of organic compounds, was measured in water treatments using the TOC-M1700 TOC analyzer in accordance with the standard method 5310B (Shimadzu, Japan). Filtration of a water sample removes sediment and other non-water components. After this first stage, the manufacturer's instructions were followed while loading the sample into the analyzer. Combustion inside the instrument converts organic carbon to carbon dioxide, which is then separated and sent to an infrared analyzer that has been set to read the concentration of Co_2. Using a calibration curve, the device's microcontroller transforms the detector signal to the organic carbon in mg/L. Spectrophotometry was used to measure the concentration of phosphate, nitrate, and nitrite (Shimadzu company UV-Vis-NIR) in accordance with the standard methods **(Lipps *et al.*, 2018)**. Throughout the experiment, pH, EC, and candidate chemical concentrations in the various water treatments were monitored every two days.

Demographic analysis

The paired bootstrap was used to evaluate the treatment differences **(Smucker, 2007; Xie *et al.*, 2021)**. The bootstrap approach with 1000 resamples was employed **(Efron & Tibshirani, 1993; Huang & Chi, 2012)**. The adult pre-oviposition period (APOP) was approximated by adding the APOP to the time it takes for an egg to hatch and then hatch into an adult, and the APOP to the time it takes for that adult to begin ovipositing. The number of days a female spends laying eggs is known as the oviposition days (O_d) **(Ning *et al.*, 2017; Xie, 2021)**. By sorting the 1000 bootstrap findings of the finite rate, the 2^{nd} and 97^{th} percentiles of life were determined.

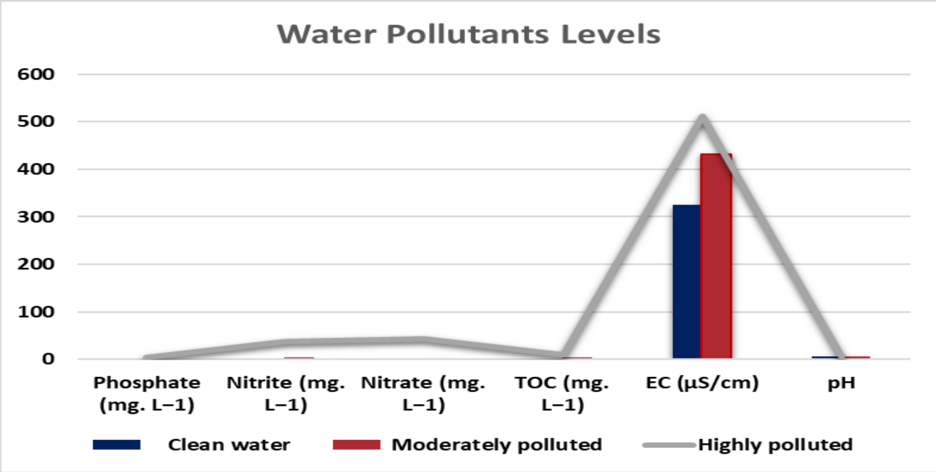

Fig. 1. Water quality: phosphate, nitrite, nitrate, and total organic carbon (TOC) concentrations, pH, and electrical conductivity (E) in clean, moderately and highly polluted water

RESULTS

Reproduction, survival, development, and the times spent in each stage by *C. pipiens'* growth in each of the three different water conditions are given in Fig. (2). The duration of time that *C. pipiens'* egg, larva, pupa, and adult stages took to mature varies depending on the water quality. The longevity of adult females was increased as immature in moderately (10.54± 0.31 days) or severely (13.63± 0.40 days) polluted water than those in clean water (9.91± 0.28 days on average). The pupal duration varied significantly across treatments. The clean water treatment had the shortest pupal duration. Total time spent in early life among treatments, however, was similar. Though, the total pre-adult duration time showed no significant difference between all treatments. In comparison to severely polluted water (0.71± 0.09), the immature survival rate in clean water (0.81± 0.02) was small and significant. The mean fertility of *C. pipiens* raised in clean water was 255.53± 12.44 eggs/female, the highest of all samples and noticeably greater than that of severely contaminated water (194.02± 18.95 eggs/ female, *P* = 0.034), however, there is no significant different on moderately contaminated water (183.92± 24.03 eggs/female, *P* = 0.018). In the clean water treatment, the number of oviposition days (Od) of *C. pipiens* took (8.31± 0.27 days) longer to complete an oviposition cycle in the clean water treatment compared to moderately and severely contaminated (Fig. 3). There was no significant change between treatments in either the APOP or the TPOP. In the clean water treatment, the average adult female adult longevity was 9.91± 0.30 days. It was substantially longer than the averages for moderately (10.54± 0.21 days) and highly (13.63± 0.72 days) polluted water. It was noticed that females live substantially longer in highly polluted water than in moderately and cleanly polluted water.

Population parameters

Finite rate of growth (λ), intrinsic growth rate (r), net reproductive rate (R_0) and mean generation time (T) are given in Table (1). In severely contaminated water, *C. pipiens* recorded values of λ, r and R_0, with 1.3116± 0.0167 d^{-1}, 0.3145± 0.0122 d^{-1} and 100.06± 15.21 offspring/individual, respectively. These numbers exceeded those seen in clean and moderately: 1.169± 0.0131 d^{-1}, 0.2047± 0.0112 d^{-1}, and 64.09 ± 9.47, 1.2311± 0.0125 d^{-1}, 0.2105± 0.0133 d^{-1} and 68.45± 13.17 offspring/individual, respectively. Remarkably, no discernible change was detected between moderately and severely contaminated water. The mean generation time (T) was not significantly different across treatments. There were no statistically significant differences in mean generation time (T), between water treatments. Out of 200 eggs, 71 female adults survived longer than 8 days ($N_{f,AL\geq8}$ = 65.03±6.12) in clean water, which was significantly higher than $N_{f,AL\geq8}$ = 49.00± 3.67 females in moderately polluted water and $N_{f,AL\geq8}$ = 46.08± 4.97 females in highly polluted water. In addition, there were more adult females living in areas of clean water compared to those living in areas with moderate or high levels of pollutants, with a value of 0.4 ($N_{f,AL\geq8}/N$ = 0.4± 0.0459, $N_{f,AL\geq8}/N$ = 0.21± 0.0262 and $N_{f,AL\geq8}/N$ = 0.24± 0.0912, respectively).

The average number of days an egg (e_{01}) survives after being laid in clean, moderately and highly polluted water treatments were 16.71 ± 0.64, 14.51 ± 0.96, and 14.26 ± 0.41 days, respectively (Fig. 2). The 1st adult female is expected to live 9.91± 0.30, 10.54± 0.21, and 13.63±0 .72 days in clean, moderately contaminated, and severly contaminated water respectively, while the first male adult is expected to live for 3.88± 0.12, 3.61± 0.17, and 4.75± 0.18 days, in clean, moderately contaminated, and highly polluted water, respectively; male adults have shorter life spans than female adults, as evidenced by their lower column.

Demographic analysis

During the increasing population, there are obvious stage overlaps. Following 30 days, it was facile to depict the total population size and its uncertainty. In the clean water, the population amounted to 61,434 individuals after 30 days. In comparison, the population in moderately contaminated water was much smaller, at 21,301 individuals, and in highly contaminated water, it was 20,299 individuals. A finite rate uncertainty is demonstrated in Fig. (4). The population parameter uncertainty for *C. pipiens* was calculated using 100,000 resamples by the bootstrap method. Around the mean, the 1000 fixed rates varied erratically (Fig. 5). The 1000 bootstrap finite rate frequency distribution of *C. pipiens* in the different aquatic environments showed a normal distribution (Fig. 6). The sorted data provided the 0.03 and 0.97 percentages of the finite growth rate. These numbers were arranged in an ascending order, which resulted in a little curve (Fig. 6).

Table 1. Mean generation time; $N_{f,AL}\geq8$, number of female adults with adult longevity≥8 days; $N_{f,AL}\geq8/N$, proportion of female adults in cohort with adult longevity≥8 days, Means (±SE) of population parameters of *Culex pipiens* reared in clean, moderately and highly polluted water: r, intrinsic rate of increase; λ, finite rate of increase; R_0, net reproductive rate; T

Parameter	Clean water	Moderately polluted	Highly polluted
λ (d^{-1})	1.3116 ± 0.0167 a	1.2311±0.0125 b	1.169±0.0131 b
r (d^{-1})	0.3145 ± 0.0122 a	0.2105±0.0133 b	0.2047±0.0112 b
T (d)	18.27 ± 0.24 a	18.16 ± 0.28 a	18.08 ± 0.23 a
R0	100.06 ± 15.21 a	68.45±13.17 b	64.09 ± 9.47 b
$N_{f,AL\geq8}/N$	59.00±4.97 a	32.00±3.67 b	35.00±6.12 b
$N_{f,AL\geq8}$	0.4±0.0912 a	2.133±0.0262 b	0.23±0.0459 b

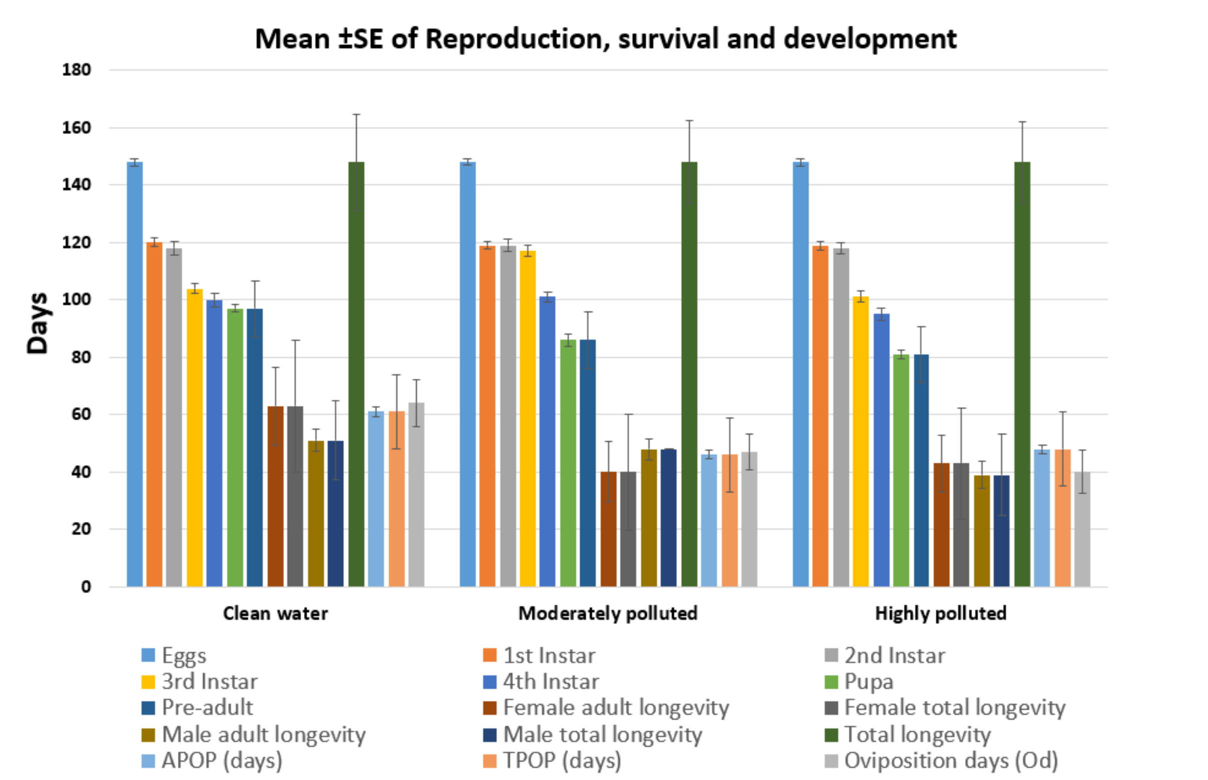

Fig. 2. Culex pipiens developmental length, lifespan, adult preoviposition period (APOP), total preoviposition period (TPOP), oviposition days (O_d) under different water treatments

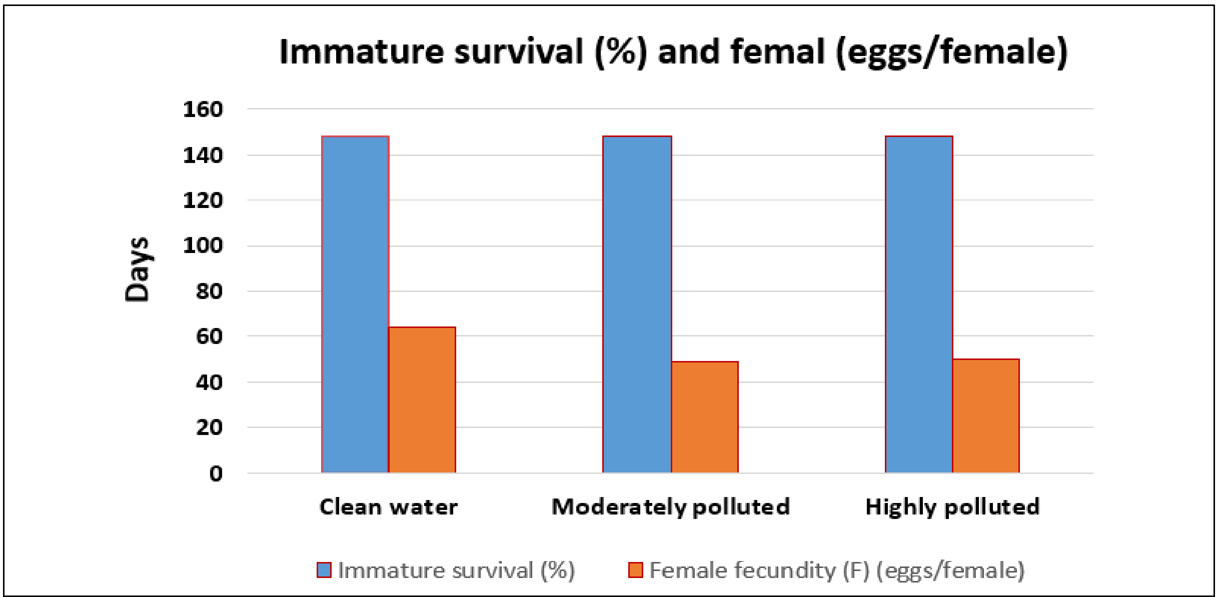

Fig. 3. Immature survival (%) and femal fecundity (eggs/female), under different water treatments

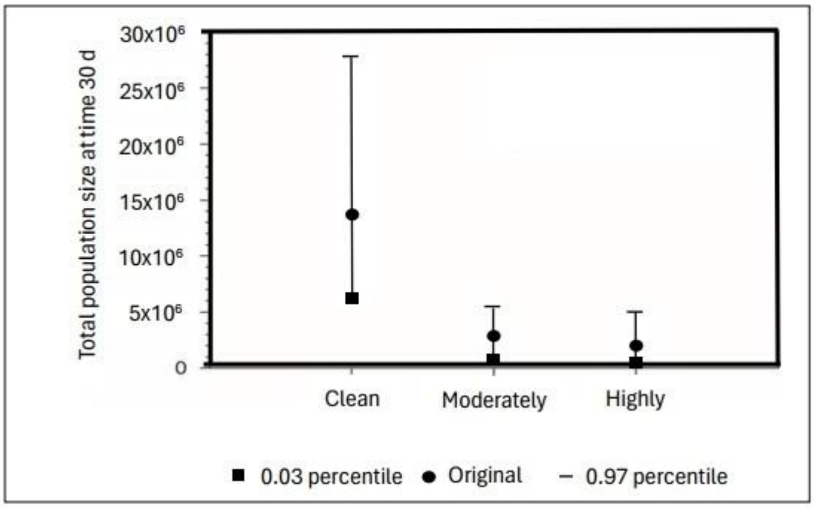

Fig. 4. Projections of population increase with the 0.03 and 0.97 in clean, moderately polluted, and highly contaminated water treatments

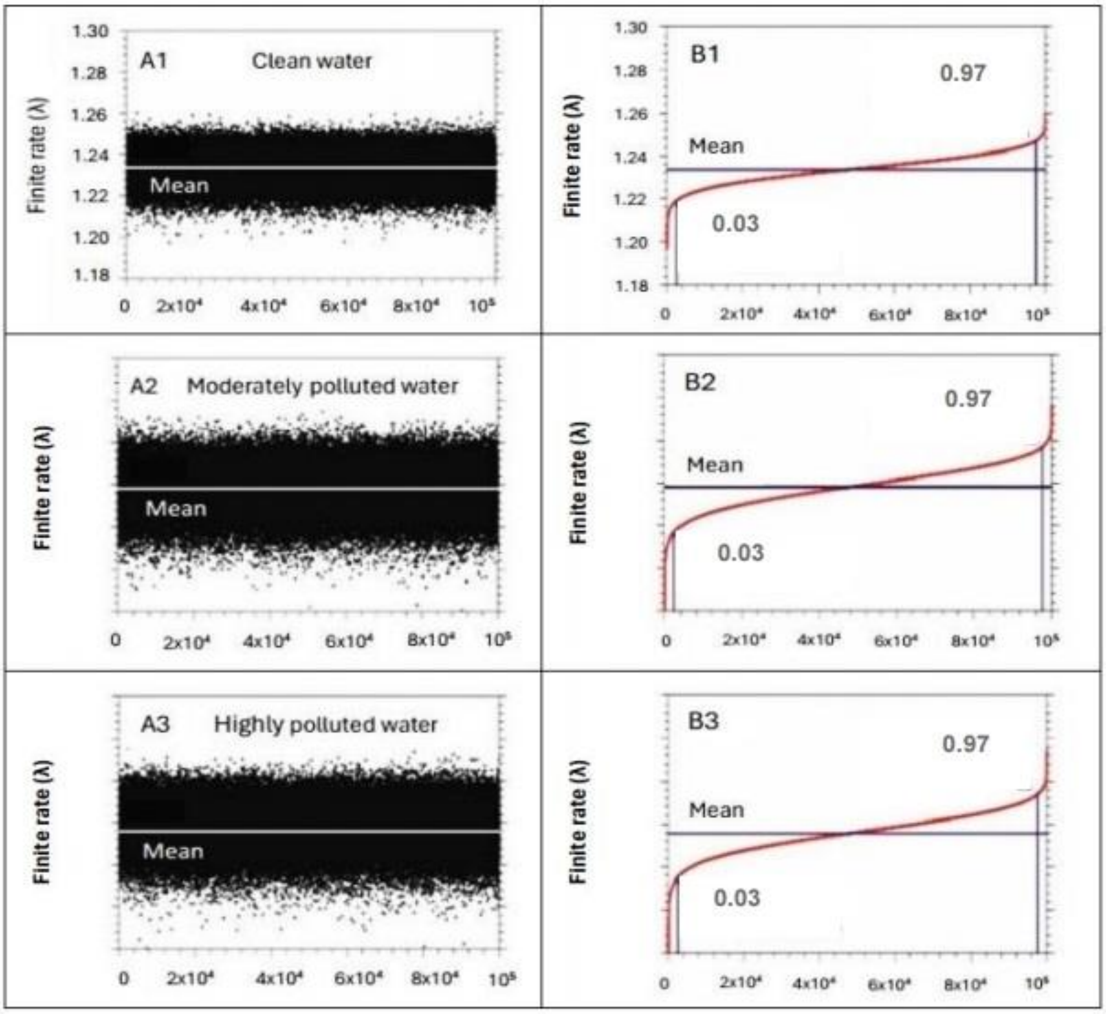

Fig. 5. Finite growth rates of 1000 bootstrap of the *Culex pipien's* in clean, moderately and highly polluted water treatments (A1– A3). The finite rates of 1000 fluctuated randomly around the mean. The finite growth rates were sorted in an ascending order, finite rates of 0.03 and 0.97 percentiles (B1– B3)

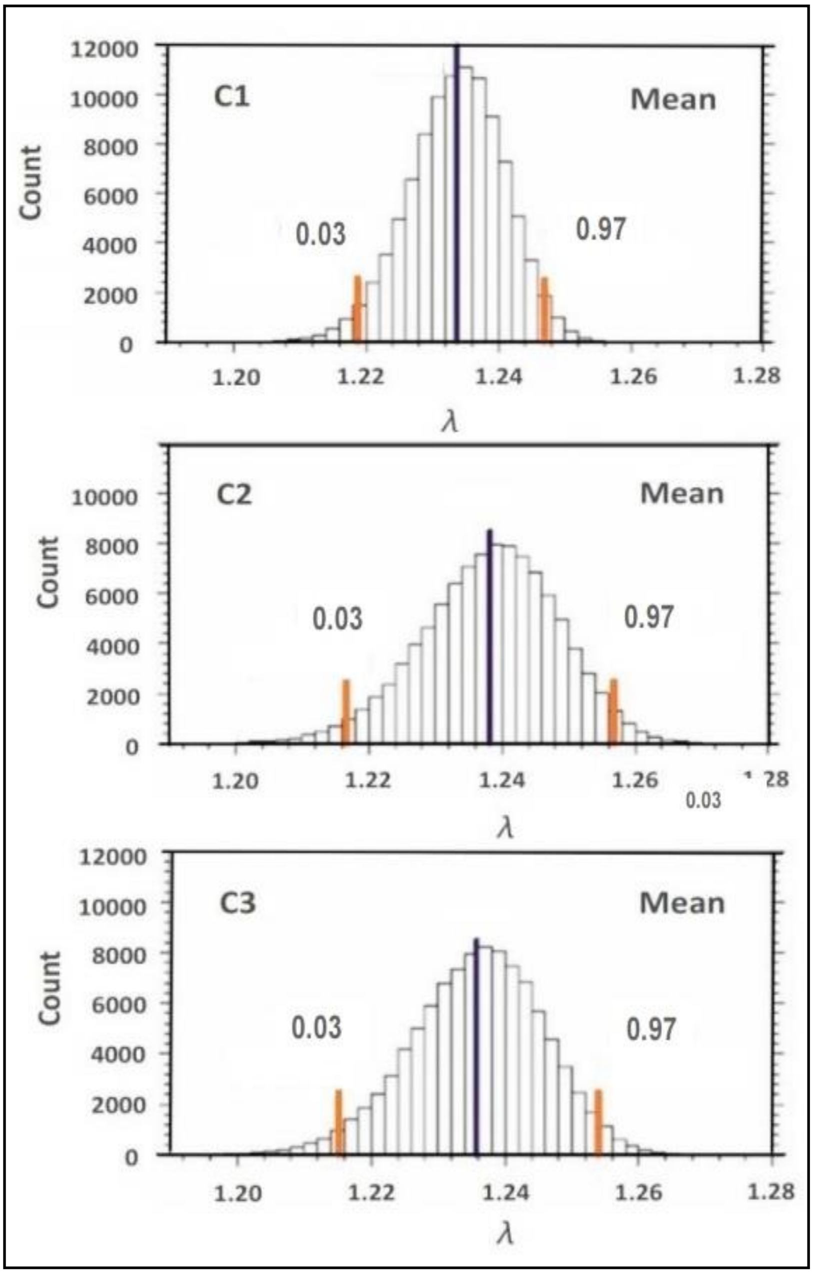

Fig. 6. Finite rates histogram of 100,000. Finite rates of 0.03 and 0.97 percentiles (C1– C3)

DISCUSSION

The impact of water pollution on the population efficiency of *C. pipiens* was discussed in this study. The filaria vector mosquito's larvae may adapt to aquatic habitats by changing their physicochemical properties. **(Edillo, 2006; Sinka *et al.*, 2010; Ndenga, 2011)**. *Culex vishnui*, *Culex tritaeniorhynchus*, *Culex bitaeniorhynchus*, *Culex quinquefasciatus*, *Culex gelidus*, *Culex fuscocephala*, and *Culex fuscanus* can adapt to a wide range of water bodies including waste or polluted waters **(Ossè, 2019; Thakare *et al.*, 2022)**.

Strategies for managing disease and vectors can be influenced by knowledge of how effectively the main filaria vectors reproduce in various aquatic habitats. It has been demonstrated that *C. pipiens* may thrive and reproduce when its eggs and larvae are raised in moderately or severely contaminated water. The Kaplan-Meier technique only provides descriptive data for the survival rate and ignores fertility, hence it provides limited information about the population efficiency. Oliver and Brooke used the Kaplan-Meier estimator and log-rank test to show that *Anopheles arabiensis* had similar adaptations in metal- contaminated water **(Oliver & Brooke, 2018)**. The overall pre-adult lifetime of *C. pipiens* did not differ significantly between clean, moderately polluted, and highly polluted water in the current study. However, female individuals that emerged from clean water had a mean lifespan that was much longer than those who emerged from moderately (10.54 d) or highly (13.63 d) polluted water. It is important to note that egg affects vector potential, because it takes time for the malaria parasite to finish developing and completing its sporogonic life cycle **(Yurttas & Alten, 2006)**.

The time that filaria parasites spend internally incubating depends on their species and the surrounding environment or the time needed for a single female to become contagious, which typically lasts 7 to 10 days. This indicates that the cohorts' individuals, at least some of them, could outlive their inherent incubation time. Female longevity varied significantly, although adult pre-oviposition (APOP) and total pre-oviposition period (TPOP) did not change. i.e. the time required to start oviposition was unaffected by water pollution. Some mosquitoes could adapt to water contaminants with cadmium chloride, copper nitrate, and lead nitrate, biological efficiency comes with a cost, including decreased egg viability, immature survivability, and decreased reproductive capabilities, according to earlier studies on *C. pipien* **(Mireji *et al.*, 2010; Oliver & Brooke, 2018)**. It was discovered that *C. pipiens* can adjust to rising phosphate, nitrate, nitrite, and TOC levels. The spread of infections is significantly restricted by mosquitoes' ability to adapt to polluted water such as malaria **(Elkington, 1993; Mireji *et al.*, 2010)**; however, it is important to consider the biological costs to mosquitoes. In order to properly calculate the biological efficiency costs for adaption, multiple population characteristics must be taken into consideration. Reduced longevity of adult females was the main cost associated with mosquitoes' adaptation to polluted water after five generations of selection, where there were fewer oviposition occurrences and fewer lifetime fertility. To combat polluted aquatic habitats, it was proposed that mosquitoes may control their biological program by altering their proteomes **(Rono *et al.*, 2019)**. Only a little research has been done on the mechanism of Culex's molecular response to water contaminants, e.g., differences in metallothionein and mucin expression can lead to the tolerance of heavy metals **(Mireji *et al.*, 2006; Rono, 2019)**. Genetic adaptation and chemical changes to aquatic systems may be possible for *Culex* species **(Mireji *et al.*, 2010)**. Additionally, prior studies demonstrated that ecological variables have an impact on the mosquito life history parameters, so they can adjust (molecular/structural) to the new circumstances that they might change **(Yurttas *et al.*, 2005; Yurttas & Alten, 2006),**

The biodiversity of aquatic species can be impacted by pollution in order to transition from naturally occurring species to species that get along **(Mireji *et al.*, 2006)**. The cost of tolerating pollutants in aquatic habitats can reduce fertility, like in this study. Being able to live close to its human hosts is important for *C. pipiens* and, as a result, it encounters chemical pollutants more frequently in prospective breeding places, especially in metropolitan areas. However, it's important to monitor the long-term effects of mosquitoes' adaptation to water pollution **(Norris & Norris, 2011)**. Given that mosquitoes' natural adversaries cannot endure pollution and thus cannot naturally reduce the mosquito population, Culex mosquitoes may adapt to polluted water and select for pesticide resistance **(Azrag & Mohammed, 2018; Jeanrenaud, 2019; Jeanrenaud *et al.*, 2020)**.

Consequently, the programs which use chemicals to manage mosquito populations may be ineffective. A huge proliferation of algae and bacteria can be produced by some pollutants, such as nitrates, nitrites, and phosphates, which are generally harmful to aquatic creatures. Culex larvae and pupae breathe directly from air through their siphons, and it does not appear to be facing a severe difficulty as a result of this **(Fried *et al.*, 2003; Ha, 2017)**. Therefore, a rise in the growth of algae and bacteria may increase the supplies of food for mosquito larvae **(Walker, 1988; Merritt, 1992; Kaufman *et al.*, 2006)**. When examining the malaria incidence rate, the significance of this issue becomes clear, and the mathematical model can evaluate its management such as "basic reproductive rate", R_0 **(Schofield *et al.*, 2007)**. This was used to assess the risk of malaria. Research investigations in natural field condition with the same or different pollutants are advised to conclude the influence of water pollutants on the risk of malaria transmission in a certain area. It should be mentioned that the current study had a number of limitations, including mosquito eggs would need to be placed singly in small rearing trays for individual mosquitoes, so the eggs may have suffered harm during this process, which may be what caused the relatively low hatching rate that was reported. Therefore, each individual L_1 stage was used in the investigation. Secondly, male and female adults could not be individually reared in cages since few females displayed a willingness to feed on blood, and those who did only produced a limited number of eggs; therefore, this part of the study was performed as a group.

CONCLUSION

The findings presented in this paper demonstrate the impact of environmental pollutants including phosphate, nitrate, nitrite, and TOC on *C. pipiens* in the laboratory. Larval stages of *Culex pipiens, Anopheles albimanus,* and *Anopheles vestitipennis* prefer unpolluted water. Nevertheless, they adjust to the conditions by paying some fitness costs in the face of the rising environmental pollution and climate change, which causes altered weather patterns such as droughts and floods. The distribution range of *Culex pipiens* may be affected by this adaptation, and the mosquito's ability to transmit disease may also vary, aspects that might have an influence on epidemiology and vector control.

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