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Life table parameters of *Anopheles stephensi* intermediate biotype in the water of four regions of Iran

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ABSTRACT

Malaria is a disease transmitted to humans through the bite of an infected female *Anopheles* mosquito. The life table is considered the most important basis for both quantitative and qualitative ecological studies, helping to understand the survival and fertility rates of insect populations. To evaluate important parameters of the life table such as life expectancy, finite population growth rate, net reproductive rate, and average generation time, the biotype of *Anopheles stephensi* intermediate was reared under laboratory conditions. Factors such as mosquito mortality rates, the number of eggs laid, the number of eggs hatched, and the male-to-female mosquito ratio were counted and analyzed. The goal of this study was to investigate important life table parameters for biotypes of *Anopheles stephensi* intermediate, in various water sources in Iran, including the semi-deep wells of Sistan and Baluchestan, the Karkheh River in Khuzestan, the Aliabad waterfall in Golestan, and the Minab River in Hormozgan, all at the National Insectarium of Iran, Pasteur Institute. The results showed significant differences between intrinsic rate of population increase (r), finite population growth rate (λ), net reproductive rate (R0), and the average generation time (T). In this biotype of mosquitoes, those feeding from the southern and eastern regions had the highest r, λ, R0, and T values. The adaptation to water from the southern regions was higher than to water from other parts of Iran. It can be concluded that water quality and adaptation to water are important factors in the growth and population of *Anopheles* mosquitoes.

Keywords:

An. stephensi intermediate, life table, water quality

1. Introduction

Malaria is a very old disease. It occurs in many parts of the world and imposes a heavy burden of complications and mortality on affected populations. Currently, Iran is in the elimination phase recognized by the WHO, but population movements across the southeastern borders are one of the main threats to the success of the malaria elimination program. In 2022, approximately 249 million people showed clinical symptoms of malaria, with 608,000 deaths recorded worldwide. In Iran, by the year 2023, 6,629 cases of malaria infection were reported (2). Demographic studies in entomology encompass topics such as life tables, reproductive models, mortality rates, and other indices of population stability. The life table is the most important basis for quantitative and qualitative ecological studies, used to understand the survival and fertility rates of insect populations. Information obtained from the life table provides a complete description of survival, growth, development, and fertility under various target conditions (3). The life table is a crucial tool for understanding insect population dynamics. By using life table data and identifying vulnerable stages of insects, the optimal timing for pest control can be determined (2).The study of insect life tables allows for the examination of various factors, such as the suitability of food types, climatic conditions (temperature, light, and humidity), the side effects of different pesticides, endosymbionts, geographical origins, and more, on the intrinsic rate of natural increase (r). Life tables and the quantitative description of populations are among the oldest and most important tools in population ecology (3).

Malaria transmission by *Anopheles* mosquitoes is influenced by their population density, which is affected by several abiotic factors in aquatic habitats. The physicochemical properties of aquatic habitats impact the survival, fertility, and development of *Anopheles* mosquitoes, playing a key role in disease prevalence (4). In a study on the life table of *Anopheles balabacensis*, a vector of *Plasmodium knowlesi* in Malaysia, life table parameters revealed that the net reproductive rate and intrinsic rate of population increase were lower than those of other *Anopheles* species. These findings could enhance the accuracy of epidemiological predictions (5). Another study examined the effect of water quality on the reproduction of *Anopheles stephensi*. Life table parameters indicated that females laid more eggs in polluted and moderately polluted water. Understanding the survival and reproductive capacity of malaria vector populations in different aquatic environments significantly aids in disease management and control strategies (6). In research on *Anopheles stephensi* as an invasive species and potential malaria vector in Sri Lanka, demographic studies identified that *An. stephensi* Mysorensis and *An. stephensi* Intermediate biotypes as the most prevalent. Using life table results, average survival, fertility rates, and finite population growth rates were determined (7). In another study conducted on water quality in mosquito habitats in northern Thailand, results from multiple regression models showed that water hardness, carbon dioxide, and water temperature were the most important predictor variables for *Anopheles* mosquito populations. Based on these findings, it can be concluded that malaria transmission depends on many ecological factors related to hydrology, which in turn affects the efficiency of vectors in disease transmission (8). A study on water quality and *Anopheles gambiae* larval tolerance in Cameroon revealed a low but significant correlation between physicochemical parameters and larval insecticide resistance (9). Over time, mosquitoes can adapt to conditions and the quality of pollution. Therefore, examining the water quality of different regions and its impact on population fluctuations can help researchers control mosquito populations that transmit diseases. In Iran, regions affected by malaria in the past have included Sistan and Baluchestan, Hormozgan, Khuzestan, and parts of Kerman and Fars provinces, as well as Gilan and Mazandaran (10).

The aim of this research is to examine important life table parameters (reproductive rate, intrinsic population growth rate, and life expectancy) for the *Anopheles stephensi* intermediate biotype and compare the average fertility and finite population growth rates across four water qualities from four geographical regions in Iran.

2. Material and Methods

2.1. Mosquito Rearing

The biotype of *Anopheles stephensi* intermediate was reared under laboratory conditions at the National Insectarium of Iran in 2023, at the Pasteur Institute (Figure 1). The mosquitoes are raised in water sourced from four regions: Chahnimeh reservoirs in Sistan and Baluchestan, the Karkheh River in Khuzestan, Aliabad waterfall in Golestan, and the Minab River in Hormozgan. The rearing process spans four generations.

Figure 1. *Anopheles stephensi* intermediate was reared under laboratory conditions at the National Insectarium, Pasteur Institute

For the study, 25 male and 25 female mosquitoes were released into breeding cages, where they are provided with a 10% sucrose solution for feeding. Two-liter water pans were used for mosquito breeding. First instar larvae (L1) are used to determine the life table. Subsequently, male and female mosquitoes were grouped in breeding cages after emerging from the pupal stage. Key parameters such as mosquito mortality rates, the number of eggs laid, hatching rates, and the male-to-female ratio were recorded and analyzed. Throughout the study, mosquitoes were maintained at a temperature of 27±2°C, relative humidity of 75±5%, and a 12:12 light-dark cycle.

2.2. Water Quality Assessment

For this study, water quality factors including total dissolved solids (TDS), electrical conductivity (EC), pH, total organic carbon (TOC), nitrate, and phosphate were measured in all three water samples. Total water hardness, representing the concentration of calcium and magnesium ions, was measured using a TDS-3 hardness meter. Water pH was assessed with an EZ-9908 pH meter. TOC levels were determined using a QBD1200 analyzer. Nitrate levels were measured with a Hanna HI 96786 photometer. Phosphate levels were measured using a Hach DR2800 spectrophotometer. The chemical properties of the water from the four regions are presented in Table 1.

Table 1. Water Quality of Four Regions: North, South, East, and West

Parameter	North	South	East	West
TDS(ppm)	335	185	208	320
EC (µS/cm)	450	391	422	501
PH	7.23	7.27	7.35	7.3
TOC (mg. L^{-1})	12	0.12	0.39	15
Phosphate (mg. L^{-1})	7	0.18	0.58	11
Nitrate (mg. L^{-1})	38	0.26	0.8	44

3. Data Analysis

3.1. Statistical Analysis

The statistical analysis to compare the means of fertility rate, finite population growth rate, reproductive rate, and life expectancy between the two biotypes is conducted using a completely randomized factorial design. To test the normality of the data distribution and the homogeneity of variances, Levene's test is used, and for testing normality, the Shapiro-Wilk test is applied.

To obtain important life table parameters such as finite population growth rate, life expectancy, net reproductive rate, and the average generation time, the number of mosquitoes alive on each day, as well as the number of eggs and larvae, are counted. Using these data, survival probabilities from birth to the onset of age x and the average number of offspring produced per mosquito on day x can be determined for the table.

To calculate the intrinsic rate of population increase (rm), the following formula is used:

$$\sum_{x=0}^{y} L_x m_x e^{-r_m x} = 1$$

To calculate the Gross Reproductive Rate (GRR), the following formula is used:

$$\sum_{x=0}^{y} m_x$$

The Net Reproductive Rate (R_0), which represents the ratio of the population size in the next generation to the population size at the start of the previous generation, is calculated using the following formula:

$$R_0 = \sum_{x=0}^{y} L_x m_x$$

To calculate life expectancy (e_x), the following formula is used:

$$e_x = \frac{T_x}{L_x}$$

To calculate the Doubling Time (DT) of a population, the following formula is used:

$$DT = \frac{ln_2}{r_m}$$

To calculate the finite population growth rate (λ), the following formula is used:

$$\lambda = e^{r_m}$$

In these equations, the following terms are defined:

- L_x is the **survival rate between two generations** which refers to the proportion of individuals that survive from one generation to the next.
- $\mathbf{T_x}$ is the average length of one generation.
- $\mathbf{m_x}$ is the average number of female offspring produced in each age group (11).

4. Results

The duration of the developmental stages of *Anopheles stephensi* intermediate feeding in four water qualities from regions in Iran is shown in Table 2. *Anopheles stephensi* intermediate completed all of its developmental stages in the four water types from different regions of Iran. Significant differences were observed in the developmental periods of first and second instar larvae, pupae, and adults across these four water sources. In the first and second instar larval stages, the western and northern Iranian water regions had the longest developmental times. This is likely due to the impact of mineral quality, electrical conductivity (EC), and water composition on the prolongation of the developmental period. In the pupal stage, the northern and western Iranian waters also significantly influenced the duration of the pupal stage. In contrast, for the adult stage, the longest developmental periods were observed in the eastern and southern Iranian waters, with 23.17[a] and 21.35[b], respectively. This may be due to the lesser dependence of adults on the water composition and their egg-laying behavior in these regions. In the developmental stages of eggs, third and fourth instar larvae, and males, no significant differences were found between the four Iranian regions. Additionally, TPOP (total population growth) showed significant differences among the four water sources in Iran. This can be attributed to the dependence of this parameter on the water conditions and water quality. However, no significant differences were found in the length of the fertility period or the pre-oviposition period across the four water sources, which may be due to the relationship of these parameters with the mosquitoes' blood-feeding behavior. Thus, it can be concluded that water quality does not affect the length of the fertility period or pre-oviposition period.

Table 2. Duration of Various Developmental Stages, Adult Lifespan, and Fertility of *Anopheles stephensi* intermediate Biotype in Four Water Quality Regions of Iran

	Egg	**First Instar Larvae**	**Second Instar Larvae**	**Third Instar Larvae**	**Fourth Instar Larvae**	**Pupae**	**Male**	**Female**	**Pre-oviposition Period of Adults**	**Total Pre-oviposition Period**	**Oviposition Duration**
Southern Iran's Water	1.18± 0.04	1.88[b]± 0.05	2.01[a]± 0.04	1.91± 0.04	2.23± 0.03	1.49[c]± 0.07	15.41± 0.13	21.35[b]± 0.37	1.62± 0.09	12.24[c]± 0.18	10.28[a]± 0.17
Northern Iran's Water	1.20± 0.03	2.02[a]± 0.04	2.17[b]± 0.05	1.93± 0.02	2.25± 0.02	1.90[a]± 0.04	14.98± 0.09	19.05[c]± 0.23	1.58± 0.12	13.00[a]± 0.12	10.98[b]± 0.06
Eastern Iran's Water	1.22± 0.02	1.91[b]± 0.02	2.08[b]± 0.03	1.92± 0.04	2.22± 0.04	1.67[b]± 05	15.27± 0.22	23.17[a]± 0.17	1.64± 0.03	12.60[b]± 0.21	10.45[a]± 0.08
Western Iran's Water	1.10± 0.03	2.05[a]± 0.04	2.25[a]± 0.05	1 .94± 0.03	2.24± 0.06	2.12[a]± 0.04	15.05± 0.24	18.79[c]± 0.22	1.53± 0.06	13.25[a]± 0.12	11.01[b]± 0.04
F	1.09	3.21	5.62	1.43	4.08	2.87	8.23	9.17	1.11	6.92	8.23
P	0.09	0.01	0.00	0.06	0.01	0.03	0.00	0.00	0.07	0.00	0.00

The analysis of life table parameters for *Anopheles stephensi* intermediate biotype showed significant differences between intrinsic population growth rate (r), finite population growth rate (λ), net reproductive rate (R_0), and the average generation time (T). In this biotype, mosquitoes feeding in the southern and eastern regions of Iran had the highest values of r, λ, R_0, and T, respectively. The highest values of r for the southern and eastern regions were 0.31 and 0.28, respectively. The highest values of R_0 for the intermediate biotype were 0.29 and 0.28 for the eastern and southern regions, respectively.

In the intermediate biotype, the adaptation to the southern water was higher than the adaptation to water from other regions of Iran. Based on these findings, it can be concluded that water quality and adaptation to it are important factors in the growth and population dynamics of *Anopheles* mosquitoes. The generation time range for the intermediate biotype was from 15.74 to 17.16 days. These results indicate that the intermediate biotype has a higher capacity for adaptation to the waters of Iranian regions, and if it migrates to other parts of Iran, it will have a greater capacity to adapt to environmental conditions.

The results of this study are consistent with those of Fazeli-Dinan and colleagues, which showed the impact of water quality on the parameters of r, λ, R_0, and T. The values for r in Fazeli-Dinan's study were 0.25, 0.23, and 0.22 for clean, semi-polluted, and polluted waters, respectively (12). Comparing our results with this study shows that, in addition to water quality, regional water adaptation can also influence the increase of r in a population. In a study by Maharaj, the effect of seasons on the values of r in *Anopheles stephensi* populations was investigated, showing that these values were 0.17 in summer, 0.08 in autumn, and 0.09 in spring (15). This suggests that seasonal variations and species type also influence the values of r in populations.

Table 3. Parameters of Intrinsic Population Growth Rate (r), Finite Population Growth Rate (λ), Net Reproductive Rate (R_0), and Average Generation Time (T) of *Anopheles stephensi* in Water from Four Regions of Iran

Water	r	λ	R_0	T
Southern Iran	$0.31^{a}±0.00$	$1.37^{a}±0.01$	$138.85^{a}±14.88$	$17.16^{a}±0.12$
Northern Iran	$0.28^{dc}±0.00$	$1.32^{bc}±0.01$	$102.08^{bc}±13.02$	$16.56^{ab}±0.24$
Eastern Iran	$0.30^{ab}±0.00$	$1.36^{a}±0.01$	$121.41^{ab}±10.76$	$17.02^{a}±0.70$
Western Iran	$0.26^{e}±0.00$	$1.30^{c}±0.02$	$96.23^{bc}±16.17$	$15.74^{bc}±0.18$

The age-stage-specific survival rates of *Anopheles stephensi* intermediate biotype are shown in Figures 2 to 5. The survival probability of each fertilized egg of *Anopheles* until age x and developmental stage j is indicated in these figures. Based on these results, it was determined that the water quality in different regions of Iran affects the age-stage survival rates. The highest survival rate in the water of the southern, northern, eastern, and western regions of Iran for the adult female stage of the intermediate biotype mosquito was 0.44, 0.32, 0.39, and 0.29, respectively. However, for males, the highest survival rates in the southern, northern, eastern, and western waters were 0.30, 0.28, 0.28, and 0.28, respectively.

The results of the age-stage-specific survival rates showed that the longest survival days for females were 37 and 33 days in the southern and eastern waters, respectively. Analysis of the survival curves revealed that the highest average survival rates for immature stages corresponded to the southern and eastern regions, as shown in Figures 2 to 5. In our study, the survival rate of females was higher than that of males. In the waters of the southern and eastern regions, the least population fluctuations were observed for adult females, males, and immature stages. However, the highest population fluctuations were seen in the western region, which influenced the survival rate of the *Anopheles* population.

Moreover, the highest average survival ratio for immature stages, according to the figures, was observed in the southern and eastern waters, respectively. In our study, the survival rate of females was higher than that of males in the *Anopheles stephensi* population, which corresponds to the findings of Fazeli-Dinan and colleagues regarding the higher age-specific survival rate of male populations compared to females (12). The reasons for these differences are related to water quality, water composition, and the pollution present in water particles, which affect the larval population and ultimately the adult stages.

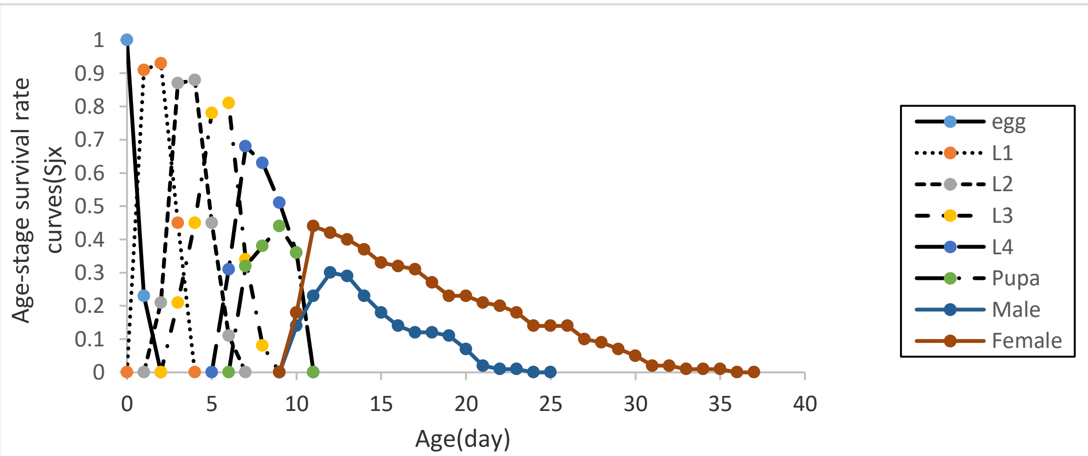

Figure 2. Age-Specific Survival Rate of *Anopheles stephensi* Intermediate Biotype in Water from the Southern Iran

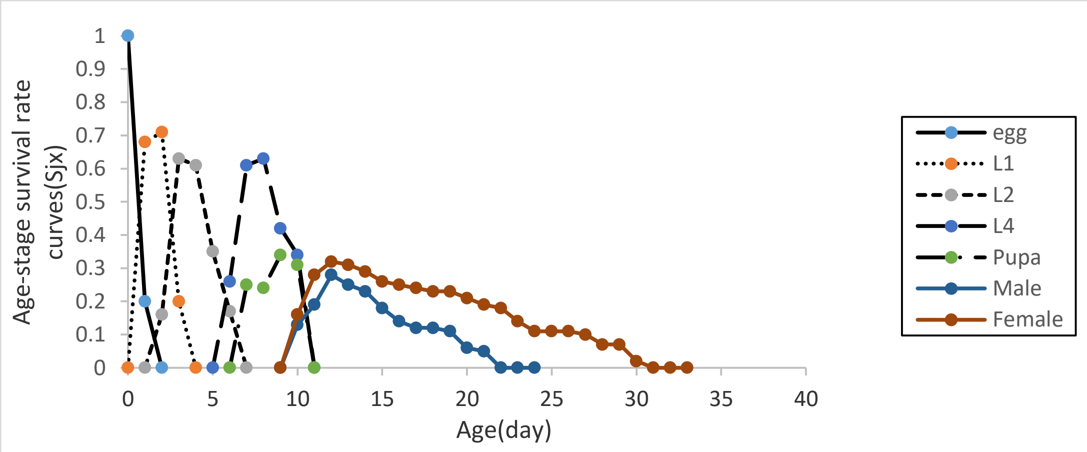

Figure 3. Age-Specific Survival Rate of *Anopheles stephensi* Intermediate Biotype in Water from the Northern Region

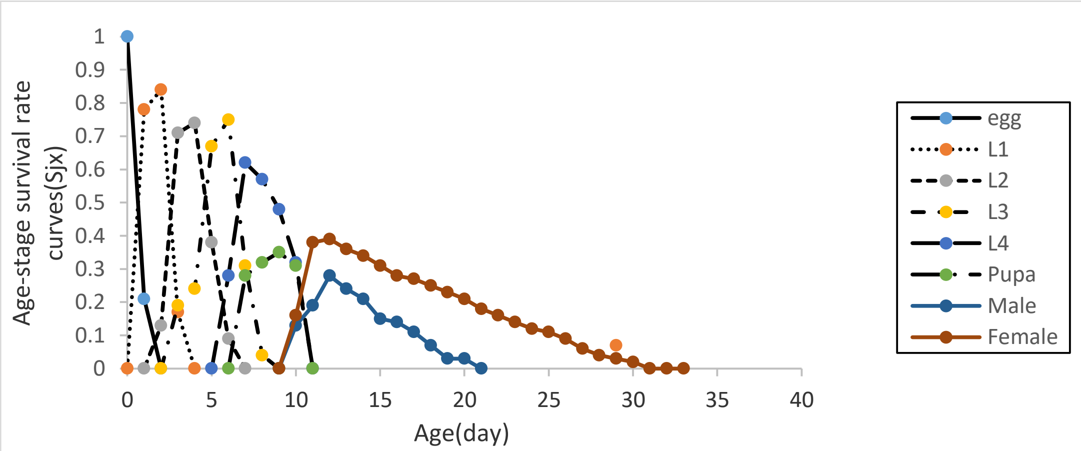

Figure 4. Age-Specific Survival Rate of *Anopheles stephensi* Intermediate Biotype in Water from the Eastern Region

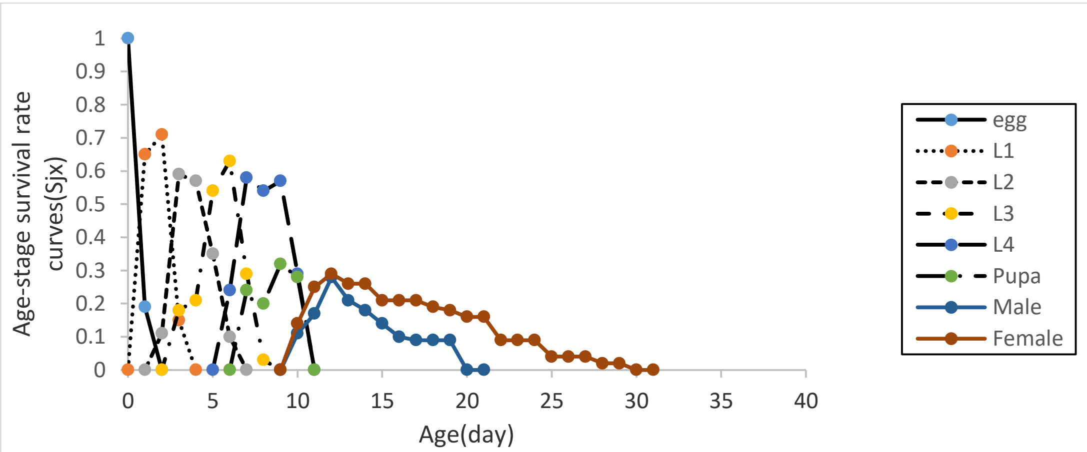

Figure 5. Age-Specific Survival Rate of *Anopheles stephensi* Intermediate Biotype in Water from the Western Region

The age-specific survival rate (lx), fertility rate (mx), and net maternity rate (lxmx) for the *Anopheles stephensi* intermediate biotype are illustrated in Figures 6 to 9. The findings indicate that the longest fertility period (mx) corresponds to water from the southern region of the country, lasting between 11 to 36 days, significantly longer than the water from other regions in Iran.

Additionally, the peaks of mx values in the southern region water exhibit the highest elevations, with the most pronounced fluctuations and peaks observed in the southern and eastern regions. Conversely, water from the western and northern regions demonstrates fewer fluctuations and lower peak values.

In the southern region's water, there is a notable decline in lx values following the onset of mx peaks, a pattern less prominent in water from the northern and western regions. Moreover, the net reproduction rate (lxmx) is significantly higher in the southern region's water compared to other regions for the intermediate biotype.

These results emphasize the influence of water quality and composition on the reproductive and survival parameters of *Anopheles stephensi*.

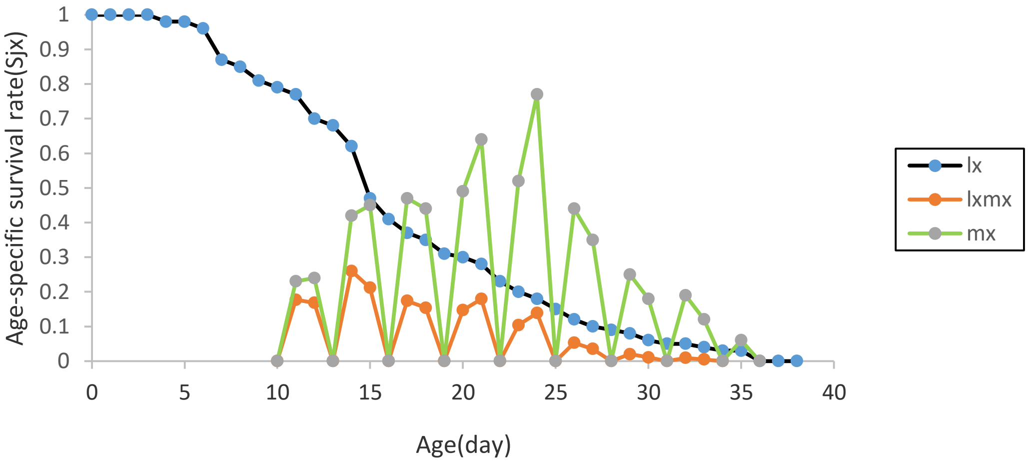

Figure 6. Survival Rate (lx), Total Fertility Rate (mx), and Net Maternity Rate (lxmx) of *Anopheles stephensi* Intermediate Biotype in Water from the Southern Region

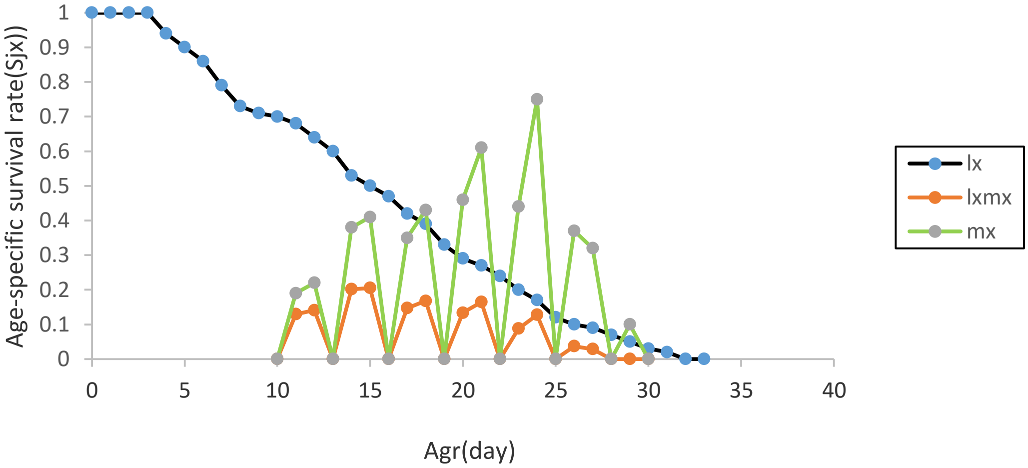

Figure 7. Survival Rate (lx), Total Fertility Rate (mx), and Net Maternity Rate (lxmx) of *Anopheles stephensi* Intermediate Biotype in Water from the Northern Region

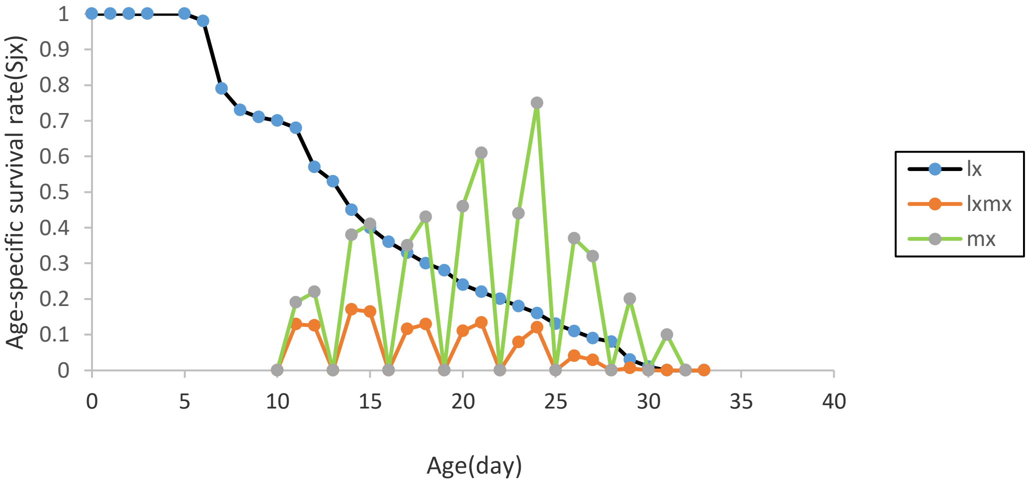

Figure 8. Survival Rate (lx), Total Fertility Rate (mx), and Net Maternity Rate (lxmx) of *Anopheles stephensi* Intermediate Biotype in Water from the Eastern Region

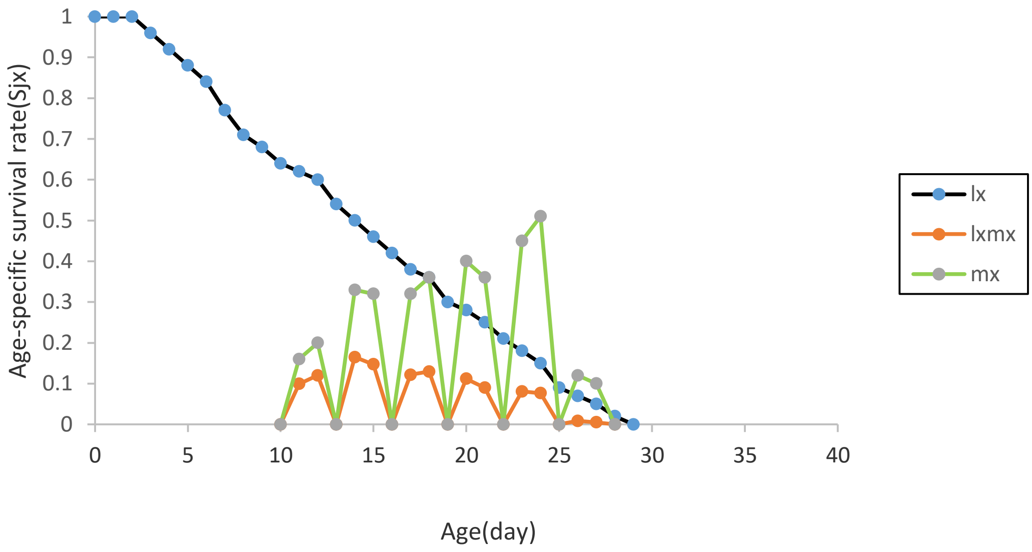

Figure 9. Survival Rate (lx), Total Fertility Rate (mx), and Net Maternity Rate (lxmx) of *Anopheles stephensi* Intermediate Biotype in Water from the Western Region

The life expectancy of age-stage groups of *Anopheles stephensi* intermediate biotype mosquitoes in southern and eastern regions was higher compared to northern and western regions. In the intermediate biotype, life expectancy during the immature stages in waters from the southern, northern, eastern, and western regions showed minimal fluctuations, with a gradual decline from the egg stage to pupation. Across all water types from different regions of Iran, the life expectancy of females was higher than that of males.

The highest life expectancy values for males were observed in waters from the southern, northern, eastern, and western regions, measuring 5.7, 6.6, 7.2, and 6.4 days, respectively. Minimal fluctuations were recorded in waters from the southern and eastern regions, while the greatest variability in life expectancy occurred in the northern and western regions. The largest difference in life expectancy between males and females was observed in the southern and eastern waters, with the smallest difference seen in the northern and western waters (Figure 10-13).

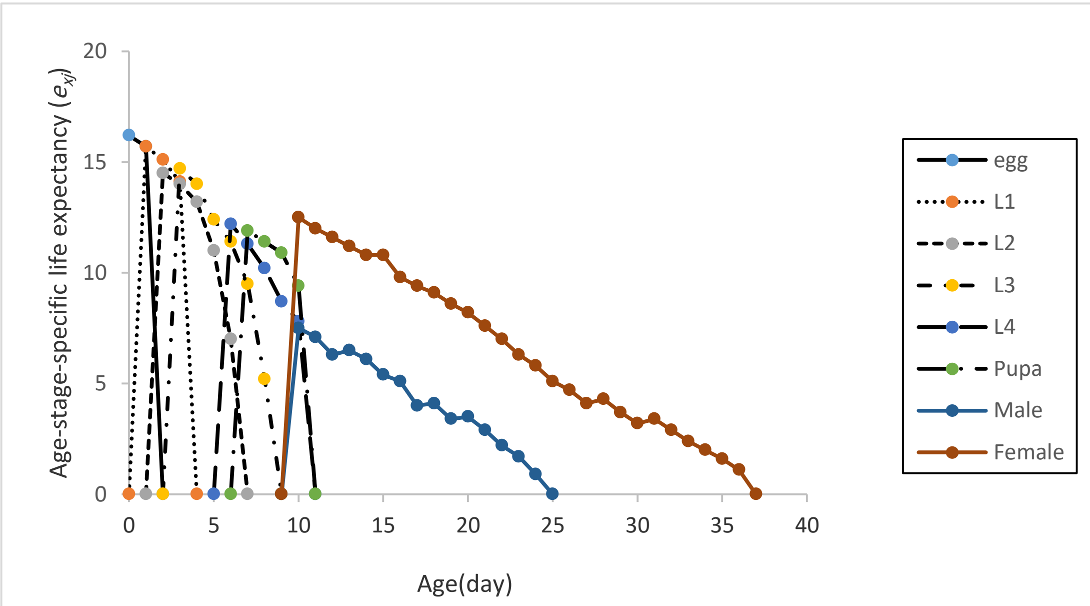

Figure 10. Life expectancy of *Anopheles stephensi* Intermediate Biotype in water from the Southern Region

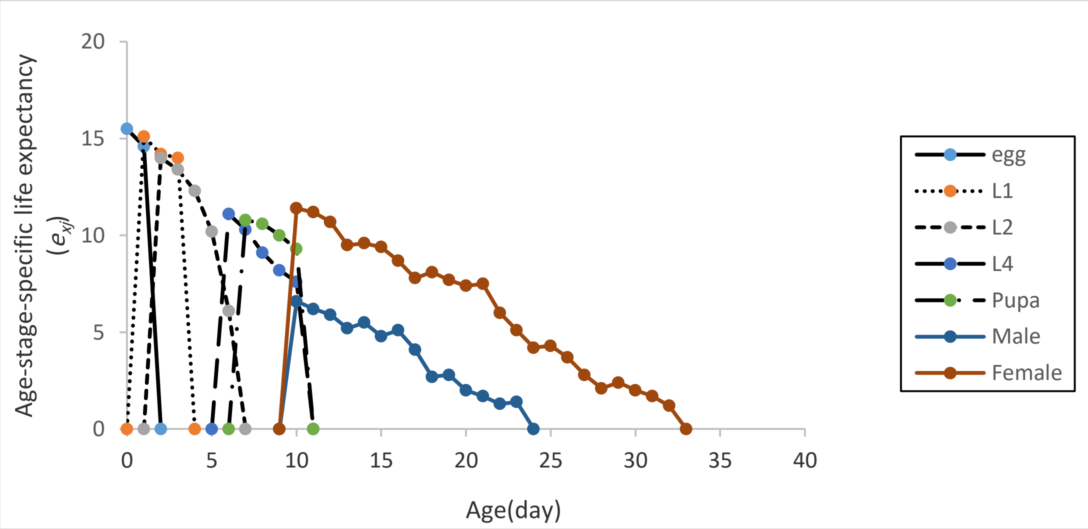

Figure 11. Life expectancy of *Anopheles stephensi* Intermediate Biotype in water from the Northern Region

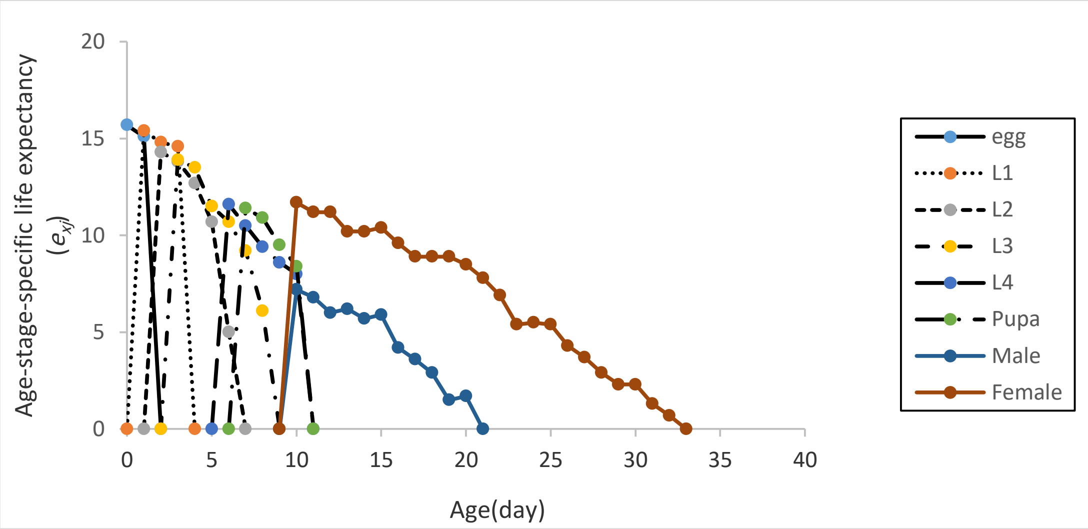

Figure 12. Life expectancy of *Anopheles stephensi* Intermediate Biotype in water from the Eastern Region

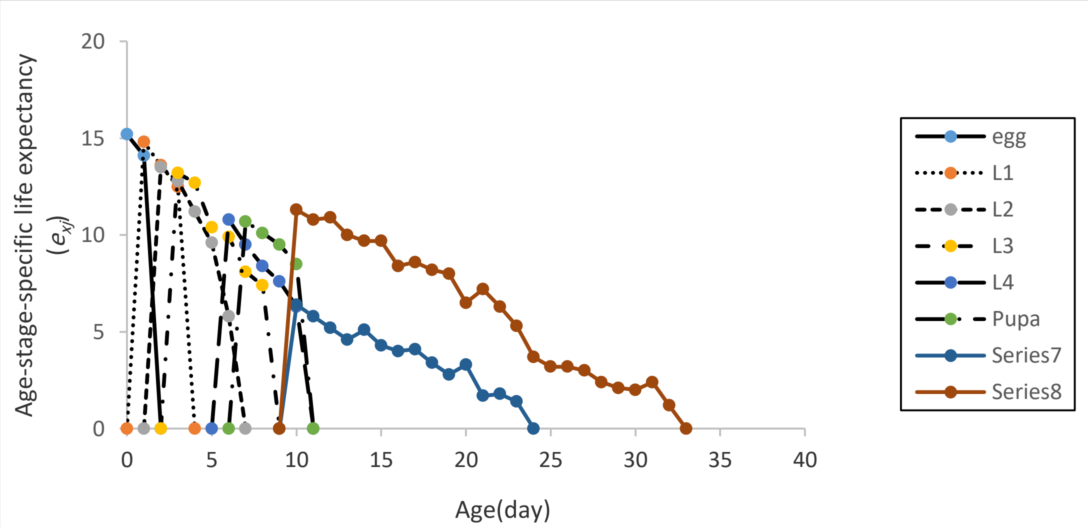

Figure 13. Life expectancy of *Anopheles stephensi* Intermediate Biotype in water from the Western Region of Iran

5. Discussion

Examining the differences in the duration of the fertility period might be attributed to the dependency of this parameter on water conditions and water quality. However, the difference in the duration of the fertility period and the pre-oviposition period in these four types of water was not significant, which might be due to the dependency of these parameters on mosquito blood-feeding. It was concluded that water quality does not affect the duration of the fertility period or the pre-oviposition period.

The results of this study were consistent with the findings of Fazeli-Dinan et al., which indicated that water quality impacts the differences in the duration of developmental stages (8). In the study by Joudeh et al., the duration of the egg stage was 1.9 days, which did not align with the findings of this study (9). Moreover, the duration of the egg stage for the species *Anopheles balabacensis* was 2 days (10), which shows a significant difference compared to *Anopheles stephensi*. However, in the study by Fazeli-Dinan et al., the average duration of the egg stage in clean water was 1.11 days, and in polluted water, it was 1.19 days (8).

In another study, the duration of the larval stages 1 to 3 of *Anopheles stephensi* was 2, 2, and 1.9 days, respectively. In the study by Chua et al., the duration of the larval stages of *Anopheles balabacensis* from stages 1 to 4 varied as 2.32, 3.69, 2.32, and 2.19 days, respectively (10). These results indicate that the physical and chemical properties of water, as well as the species type, influence the duration of developmental stages.

In our study, the survival rate of female mosquitoes was higher than that of male *Anopheles stephensi* mosquitoes, which was consistent with the findings of Fazeli-Dinan et al., who reported a higher age-specific survival rate for males compared to females. The reasons for these differences are linked to water quality, water composition, and the pollution present in water particles, which affect the larval population and ultimately the adult stages.

6. Conclusion

The results of this study showed that water quality plays a very important role in the reproduction and population of *Anopheles* mosquitoes, including *Anopheles stephensi*. Water quality creates favorable or unfavorable conditions for the growth of larvae and egg-laying. *Anopheles stephensi* has a strong preference for laying eggs in clear water, with the water in the southern and eastern regions having higher clarity compared to the water in the northern and western regions. Water with lower TOC, such as that in the southern and eastern parts of Iran, is more suitable for egg-laying and larval development than the water in the northern and western regions with higher TOC. High hardness waters are not suitable for egg-laying and larval development. Overall, the results suggest that the management of stagnant, clear waters with low hardness and lower electrical conductivity should be prioritized. Additionally, the adaptability of *Anopheles stephensi* strains should be evaluated to enable targeted mosquito population control as a health measure.

Conflict of Interest

There is no conflict of interest.

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