

Laboratory studies on the Effect of different temperatures on *Biomphalaria alexandrina* snails with emphasis on the consequences of global warming

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ABSTRACT

The objective of the present study was to examine the impact of increasing water temperatures on the survival, growth rate, and reproduction of *Biomphalaria alexandrina* snails, as well as their susceptibility to *Schistosoma mansoni* infection. The study examined a range of water temperatures, including 30, 32, and 34°C, and a control group maintained at room temperature. The results indicated that increasing water temperature significantly reduces the survival rate of *B. alexandrina* snails. The survival rate was 0, 6, 9, and 91% for snail groups at 34, 32, 30 °C, and room temperature, respectively. In contrast, there was no statistical difference between increased temperature (30 and 32 °C) and room temperature on the growth rate of the snails. The mean shell diameters of snails were 9.8 and 9.6 mm at 30 and 32 °C, respectively, compared to 10.03 mm at room temperature. However, the growth rate of snails at 34°C reached 8.4 mm. Increasing water temperature dramatically suppressed the reproduction rate, resulting in fewer egg masses. Snails maintained at room temperature laid eggs in the tenth week and continued to lay eggs till the end of the experiment (14th week). Those exposed to the water temperature of 30 °C were observed to lay eggs in the tenth week; however, after two weeks, they stopped laying. There was no egg laying for the snails exposed to 32 and 34°C throughout the experimental period. This was confirmed by the histological examinations of the hermaphrodite gland, which revealed damage in the gonadal cells, ranging from mild to severe, according to the increase in water temperature. The infection rate of infected snails decreased significantly by increasing the temperature. The total number of produced cercaria decreased sharply by increasing the water temperature. The infected snails produced 2222, 634, and 265 cercariae at 25, 30, and 32°C, respectively. However, at 34 °C, no cercariae were detected in the snails.

INTRODUCTION

Global warming is defined as an increase in the temperature. It can be interpreted as changes in climate caused by natural phenomena or human activities such as greenhouse gases (Zhao *et al.*, 2020). The consequences of these changes relating to human health are still being studied, with an increased interest in the incidence of infectious parasitic diseases. This is due to the sensitivity of biological systems to climate variables such as temperature, precipitation, and humidity (McCreesh & Booth, 2013). The relationship between climate

change and parasitism could alter the prevalence and intensity of existing infections in addition to the possibility of incidence of new infections in the geographical distribution (Adekiya et al., 2020; Singh et al., 2020).

Schistosomiasis is a parasitic disease in Egypt. It is caused by trematode *Schistosoma mansoni*. The transmission of this disease is related to the presence of *Biomphalaria alexandrina* snails, which act as intermediate hosts (Jarne et al., 2011). Climatic factors, especially temperature and precipitation, exert a strong influence on the prevalence of the disease. In scenarios with climate change, where an increased environmental temperature is noticed, the epidemiology of schistosomiasis will be directly influenced (Paull & Johnson, 2011). Understanding the patterns of disease transmission in their habitats and how the mollusk parasite relationship responds to temperature variability would help develop effective control programs against this disease.

The temperature rise may alter the optimal conditions for snail breeding, growth, and survival, eventually affecting schistosomiasis transmission. Also, climate change may affect the population size of snails, parasite density, and disease epidemiology.

Hence, the principal aim of this investigation was to assess the influence of elevated water temperatures (specifically 30, 32, and 34°C) on the survival, growth rate, and reproductive capacity of *B. alexandrina* snails, as well as their susceptibility to *S. mansoni* infection. This knowledge can be utilized to implement proactive measures to mitigate schistosomiasis transmission in Egypt.

MATERIALS AND METHODS

The present investigation assessed the impacts of different water temperatures, especially 30, 32, and 34 °C. Two separate experiments were carried out, one to assess the impact of increased water temperatures on the biological traits of *B. alexandrina* snails and the other to examine the effects of these temperatures on the vulnerability of these snails to *S. mansoni* infection.

1. *Biomphalaria alexandrina* snails

B. alexandrina snails were chosen as the focal species for this study due to their high prevalence in the Nile Delta region of Egypt (El Khayat et al., 2009; El-Khayat et al., 2013; El-Khayat et al., 2017; El-Zeiny et al., 2021).

480 snails, measuring 4-6 mm, were purchased from the Schistosomiasis Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI). They were housed in glass containers with a capacity of 1 L and subjected to laboratory conditions for a duration of roughly 20 generations in the absence of any external parasite pressure. These containers were filled with dechlorinated tap water and were equipped with a perforated plastic top to

ensure that the snails remained within their respective aquaria and did not escape. The snails were fed a diet of dried lettuce powder (*Lactuca sativa L*) twice weekly. In order to mitigate the risk of bacterial proliferation and contamination, the water was subjected to weekly renewal.

2. Experimental design

Four experimental sets were prepared. Three sets were designed for three different water temperatures adjusted for 30, 32, and 34 °C using water baths. The fourth set was exposed to room temperature (RT).

480 snails (4–5 mm) were used throughout the study (two experiments).

- The first experiment (14 weeks) was designed to assess the biological and histological effects of increasing water temperatures. Six replicates of 15 snails (n=90) were used for each temperature set.
- In the second experiment (6 weeks), 3 replicates of 10 snails (n=30) were used in each temperature set. This experiment was conducted to examine the effects of increasing water temperatures on the vulnerability of these snails to *S. mansoni* infection.

The water temperatures at the room conditions were recorded daily. The overall mean water temperature in the room was 21±5.95 °C in the first experiment and 25±1.81 °C in the second experiment; they were not conducted at the same time.

3. Biological and histological investigations:

3.1. Survival rate of snails:

The survivorship of the four distinct sets was observed daily. During the study, the quantity of living and dead snails was carefully documented, with the latter being promptly eliminated to maintain water cleanliness and mitigate bacterial proliferation. The snails' death was verified by identifying distinct odors associated with decomposed organic material and the absence of any reaction to delicate stimulation using a needle (**Oteifa et al., 1975**). The study used **Frank's (1963)** equation to compute the survival rate as follows;

$$\text{Survival rate / week} = \frac{\text{Number of alive snails}}{\text{Total number of used snails}} \times 100$$

3.2. Growth rate of snails:

The present investigation involved observing the growth of juvenile snails (n=360) within a size range of 4-5 mm. The study followed a weekly monitoring approach, where the shell diameter of each snail was measured in millimeters to track their developmental progress, as outlined by **Chernin and Michelson (1957)**.

3.3. Reproduction of snails:

The adult snails in the experimental aquaria were observed daily to determine their egg-laying behavior. This was achieved by providing plastic sheets specifically designed for oviposition. Subsequently, the egg masses were gathered.

Each week, egg masses and snails were quantified using a hand lens with a magnification of 10x. Weekly documentation was conducted to record the cumulative count of egg masses deposited by both the treated and control snails.

The egg-laying capacity refers to the quantity of eggs produced each week by a population of snails (Mx). This metric is determined by dividing the number of eggs laid during a given week by the total number of snails alive at the start of that week (**Baz *et al.*, 2022**).

3.4. Histology of the hermaphrodite gland:

The study examined the prospective histological changes in the hermaphrodite gland of snails exposed to water temperatures of 30, 32, and 34°C and those maintained in water at 25°C (control). Five snails were chosen at random from each experimental group. After crushing the shells meticulously, fragments were removed with care using a dissecting microscope. Following the dissection of snails, the hermaphrodite glands were examined using the standard protocol outlined by **Slaoui and Fiette (2011)** and **Ismail *et al.* (2013)**. The specimens were then subjected to microscopic examinations using a light microscope, and images were acquired using a microscopic digital camera.

4. Infection with *Schistosoma mansoni*:

4.1. Exposure to miracidia:

In this experiment, snails (4-6 mm) were exposed to *S. mansoni* miracidia at water temperatures of 25°C (control), 30, 32, and 34 °C.

S. mansoni miracidia were obtained from SBSC-TBRI. They were freshly emerging (within one hour) and showed active and vital motion. As an individual infection protocol, Snails were exposed individually to 6-8 miracidia of *S. mansoni* for 24 h in multiwell plates filled with 2 ml of dechlorinated tap water/each well (**Anderson *et al.*, 1982**).

4.2. Cercarial shedding:

After 10 days post-infection, surviving snails from each group were individually examined in multiwell plates for cercarial discharge. The snails were exposed to light (desk lamp) for one hour to hasten the discharge of cercaria (**Meuleman, 1972**). Positively infected snails (shedding) were transferred to sterile aquaria containing dechlorinated water and maintained at the same temperature and in a dark location. A few droplets of Bouin's fluid

were used to stain the cercariae shed by each snail to make their counting under a stereomicroscope simpler. This inspection was conducted weekly to prevent mollusk fatigue.

The time between a snail's exposure to miracidia and the first shedding of cercariae is known as the incubation period (prepatent period).

The number of cercariae generated by each snail was measured as the number of cercariae shed per snail per week for all snails that shed cercariae. The total number of cercariae produced was then calculated by adding the total number of cercariae produced by all snails during the experimental period.

$$\text{Total number of cercarial production} = \Sigma \text{ cercariae shed/snail/week}$$

The survival rate was calculated by dividing the number of survived snails at the first shedding by the total number of exposed snails at the beginning of the experiment.

$$\text{Survival rate} = \frac{\text{Number of survived snails at 1}^{\text{st}} \text{ shedding}}{\text{Number of exposed snails}} \times 100$$

The infection rate of snails was calculated by dividing the number of infected snails by the number of survived snails at the first shedding time according to **Coles (1973)** by the following equation:

$$\text{Infection rate} = \frac{\text{Number of infected snails}}{\text{Number of survived snails at first shedding}} \times 100$$

5. Statistical analysis:

GraphPad Prism 8 was used to conduct statistical analysis. The data was categorized based on the various temperatures. Using the Kolmogorov-Smirnov test, the data were examined for normal distribution. If the data had a normal distribution, the ANOVA test was conducted. A one-way ANOVA was used to compare the four distinct water temperature groups. Combining ANOVA with Tukey's post hoc test allowed for multiple comparisons across all water temperature categories. The number of egg masses and the number of eggs were compared between the room temperature and 30 °C groups using a T-test. The infection rate results were presented as percentages and analyzed with chi-square values derived from contingency tables. The difference between groups is statistically significant when the *P*-value is less than 0.05.

RESULTS AND DISCUSSION

1. Biological and histological investigations

1.1. The survival rate of snails:

The survival rate of snails exposed to the highest temperature (34 °C) decreased gradually with time till a sharp decrease in the 3rd week when the survival rate reached 64% and then continued to decrease till all snails were dead in the 10th week. Snails at 32 °C showed a similar pattern of survival rate where a sharp decrease was observed in the fourth week, and their death extended with time until only 6% of snails were alive at the end of the experiment (14 weeks). At 30 °C, snails seemed to be adapted to this temperature for a long time (4 weeks) with a high survival rate (91 %), but some snails could not stand up more, and their survival rate decreased suddenly at the 5th week and continued in its reduction till 9% survival at 14th week. On the other hand, snails maintained at room temperature (21°C) were exposed to conditions similar to natural conditions with ups and downs in the surrounding temperature. This group showed the highest survival rate during the study period, with an end survival rate of 63% after a 14-week experiment.

In other words, the threshold of the survival period is 3 weeks for snail groups at 34 °C, 4 weeks at 32 °C, and 5 weeks at 30 °C (**Figure 1**).

The survival rates of the snails exposed to 30, 32, and 34 °C and the snail group maintained at room temperature differed significantly with a *P*-value of 0.002. Snails exposed to 32 and 34 °C differed significantly from those maintained at room temperature (*P*-value; 0.018 and 0.002, respectively). However, the survival rate of snails exposed to 30 °C didn't vary significantly from any other groups with *P*-values of 0.11, 0.88, and 0.43 versus the survival rate of the snails at room temperature, 32, and 34 °C groups, respectively. Furthermore, the results show that the survival rate of snails exposed to 32 °C is statistically (*P*-value 0.86) as low as that of snails exposed to 34 °C. So, it is obvious that only snails maintained at room temperature showed the highest survival rate .

In the current study, it was obvious that the survival rate of *B. alexandrina* snails significantly decreased by the higher temperatures. These results agree with many authors' previous reports (**El-Emam & Madsen, 1982; Kubirizajournal et al., 2010; McCreesh et al., 2014; Kalinda et al., 2017**). In Egypt, **El-Emam and Madsen (1982)** found that the survival of *B. alexandrina* has greatly reduced at 33 °C compared to 25, 26, and 28 °C. Similarly, **Kubirizajournal et al. (2010)** reported no significant differences in the survival rate of snails at 25, 28, and 31 °C. However, they added that it was optimal at 25°C. Moreover, **McCreesh et al. (2014)** stated that there was very strong evidence that water temperature had a significant effect on snail mortality rates (*P* < 0.0001) of *B. sudanica*. Among the different water temperatures tested in their study; 15°C, 19°C, 23°C, 27°C, and

31°C, the snail survival was estimated to be highest at 19.9°C. **Kalinda et al. (2017)** observed that the survival of *B. alexandrina* is reduced at temperatures above 33 °C.

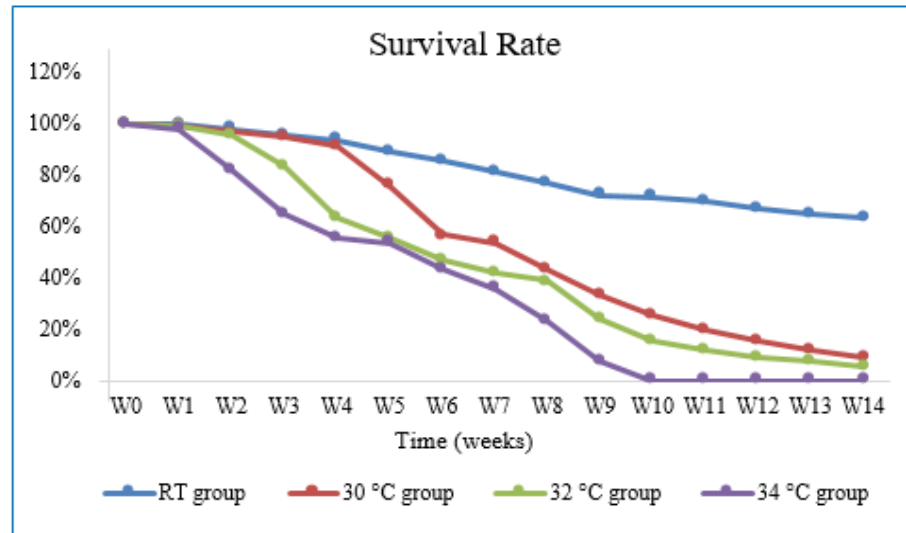


Fig.1. Survival rate of *Biomphalaria alexandrina* snails at different water temperatures

1.2. The growth rate of snails:

Figure 2 illustrates the growth rate of *B. alexandrina* snails across different experimental groups. No significant difference was observed in the growth rate of snails when exposed to water temperatures of 30 and 32 °C compared to those reared at 21°C (room temperature). The snails cultivated at 34°C exhibited a significantly reduced growth rate compared to those subjected to a temperature of 21°C. Upon conclusion of the experiment, specifically in the 14th week, the mean shell diameter of snails was observed to be 9.8 mm and 9.6 mm at temperatures of 30°C and 32°C, respectively. In comparison, the average shell diameter at a temperature of 21°C was recorded as 10.03 mm. On the other hand, the growth rate of the specimens kept in water at a temperature of 34°C reached 8.4 mm by the ninth week before their death.

Overall, the increase in water temperature did not significantly impact the growth rate of *B. alexandrina*. This finding aligns with the research conducted by **El-Emam and Madsen (1982)**, which also concluded that there were no significant differences in the growth of snails maintained at temperatures of 25, 28, and 31 °C. According to **Sturrock's (1966)** findings, it was observed that *B. pfeifferi* exhibited enhanced growth rates at a temperature of 30 °C. However, it should be noted that a decrease in survival rates accompanied this favorable growth condition

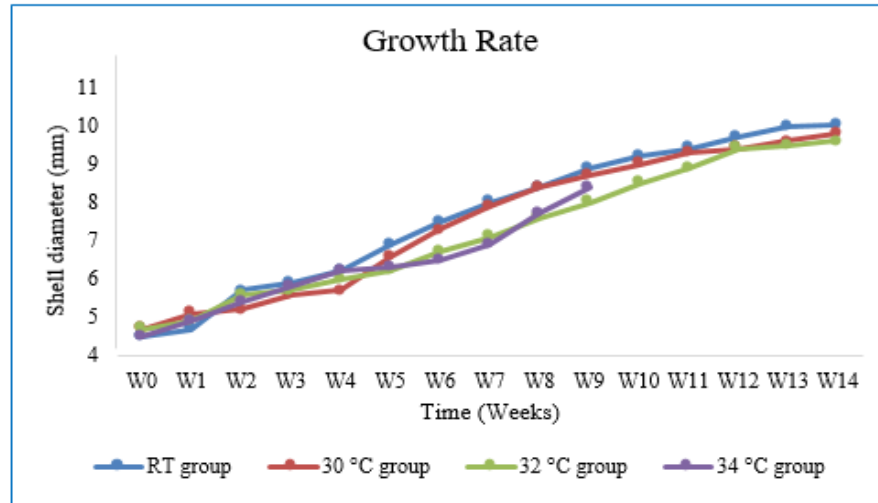


Fig.2. Growth rate of *Biomphalaria alexandrina* snails at different water temperatures

1.3. Reproduction of snails:

Results showed that the snails maintained at 21°C laid eggs in the tenth week and continued to lay eggs till the end of the experiment (14th week). Those exposed to the water temperature of 30 °C were observed to lay eggs in the tenth week and then stopped laying after two weeks from the first time of their oviposition. On the other hand, the egg-laying was inhibited for the snails exposed to 32 and 34°C throughout the experimental period (**Figure 3 A**). The results indicate that the fecundity of snails maintained at 21°C differed significantly from those exposed to the water temperature of 30 °C (**Figure 3 B**).

Regarding the reproduction of *B. alexandrina* snails, the current work revealed that higher temperatures highly affected them. As the water temperatures increased, the egg-laying reduced (and hence the reproduction rate). This confirms the results of **El-Emam and Madsen (1982)**, who observed that the net reproductive rate was optimum at 26 °C for *B. alexandrina* snails. Similar findings were obtained by **Appleton and Eriksson (1984)** in their study on *B. Pfeifferi* snails to investigate the impact of above-optimal temperatures on the fecundity of the snails. They discovered that their fecundity decreased when snails were exposed to temperatures above 27 °C. In addition, **Barbosa et al. (1987)** also stated that the reproduction rate was at its highest during autumn, when the temperature was around 22 °C. In contrast, the snail's reproductive rate changed inversely with temperature, declining as the temperature rose to 24 °C during the summer. Also, **McCreesh et al. (2014)**, in their study on *B. sudanica* at 15°C, 19°C, 23°C, 27°C, and 31°C, found very strong evidence that water temperature influenced snail fecundity. This is due to a reduction in the average number of eggs laid per snail and the average number of eggs laid per egg mass. Moreover, **Kalinda et al. (2017)** recorded that the egg-laying and the egg mass production of *B. pfeifferi* reduced above 27 °C. However, **Kubirizajournal et al. (2010)** documented little change in the average number of eggs laid at different temperatures. Comparing the net reproduction rate at 22 °C to the other three temperatures (25, 28, and 31 °C), it was much lower.

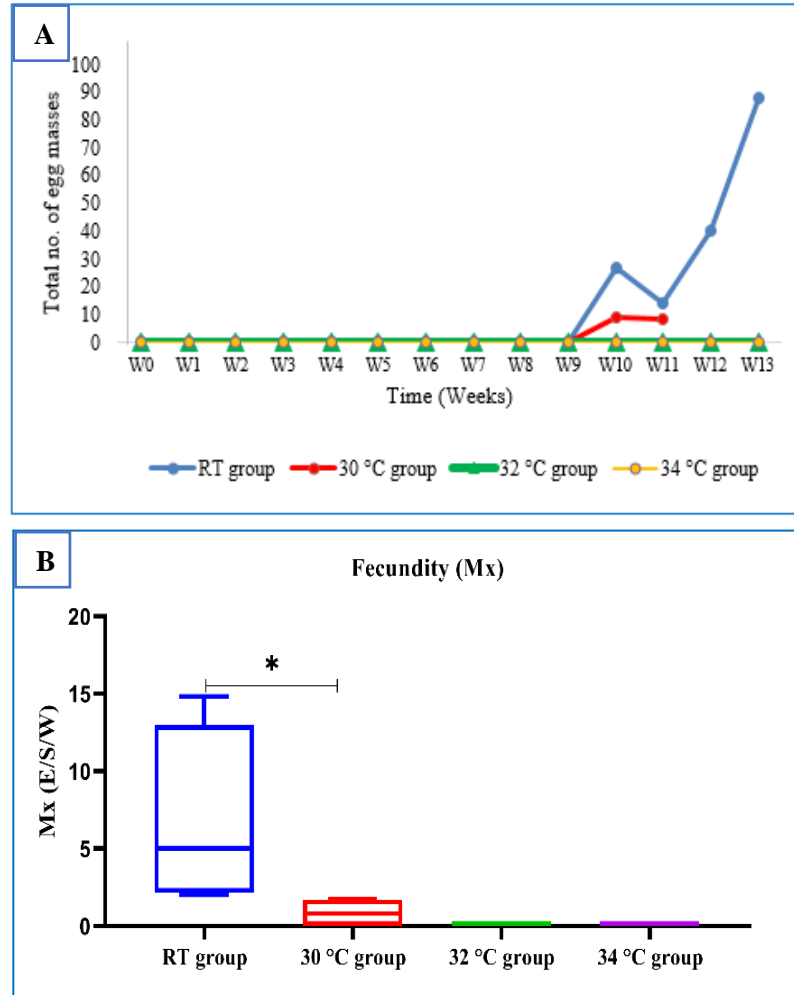


Figure 3. A: Egg-laying of *Biomphalaria alexandrina* at different water temperatures, **B:** Fecundity of *Biomphalaria alexandrina* snails at different water temperatures

1.4. Histology of the hermaphrodite gland:

The normal hermaphrodite gland of *B. alexandrina* consists of many acini that are connected with connective tissue. The acini are lined with a layer of epithelial cells that develop into male and female gametogenic cells, "spermatogenesis and oogenesis." The male cells are differentiated, forming primary and secondary spermatocytes. The female oogonia cells are usually found in groups arranged along the periphery of the acinus as primary, secondary oocytes, and mature ova. Developed sperms are free in the acinus lumen; each has a small oval head and a thread-like long tail. One acinus may contain 1-2 mature ova and sperms aggregated in large numbers inside the lumen (Mohamed *et al.*, 2010).

The present study demonstrated that there are histological changes in the hermaphrodite gland, and these changes increased by increasing the water temperatures.

At 34 °C, the effects showed marked morphological changes in both male and female gonadal cells. Most of the spermatogenesis stages were lysis, and spermatocytes became scattered. In addition, mature ova and secondary oocytes suffered from disintegration and deformation (**Figure 4; D**). The effects showed fewer morphological changes at the water temperature of 30 and 32 °C (**Figure 4; B & C**). On the other hand, the snail group reared at room temperature (21 °C) showed a good case of the hermaphrodite gland, reflecting a good reproduction state (**Figure 4; A**).

It is noteworthy to emphasize that this study represents the initial investigation into the impacts of elevated temperatures on the hermaphrodite gland. The current results were similar to those who studied the impact of different molluscicides on that gland, which means that warmer temperatures could induce cellular damage similar to molluscicides. **Osman *et al.* (2008)** found that exposure of *B. alexandrina* snails to sublethal concentrations of the herbicides Roundup and/or Topik showed complete destruction of gametogenic cells. Also, the present results matched the finding of **Mossalem *et al.* (2013)** who studied the molluscicidal properties of the anti-helminthic plant derivative, dihydro-artemisinin methyl ether (artemether) against some histological and histochemical parameters of *B. alexandrina* snails and stated that there is complete destruction of gametogenic cells and severe damage of hermaphrodite gland tissues, especially when the exposure period increased. Similar observations were mentioned by **Ismail *et al.* (2013)** in their study on the effect of the molluscicidal activity of lemon salt (citric acid) against *B. alexandrina* snails. They found that there was a gradual marked reduction in oogenesis and spermatogenesis. Furthermore, the connective tissues degenerated after 24, 48 hours, and one week of exposure to the tested compound. The present results coincide with the findings by **Abdel-Ghaffar *et al.* (2016)**, where the histological examination of the hermaphrodite gland in treated snails with glyphosate isopropyl ammonium and pendimethalin herbicides showed loss of connective tissue, irregular sperm, and mature ova appeared dense and irregular in shape and some degenerate.

Thus, the effect of different water temperatures on the egg-laying capacity of *B. alexandrina* in the present study might be due to its effect on the sexual organs of the snails (gametogenic cells). These data confirm the present investigations on the histological structure of the hermaphrodite gland of snails, which indicated a strong correlation between the lower capacities of egg-laying of the snails exposed to higher temperatures (32 and 34°C) and hermaphrodite gland changes.

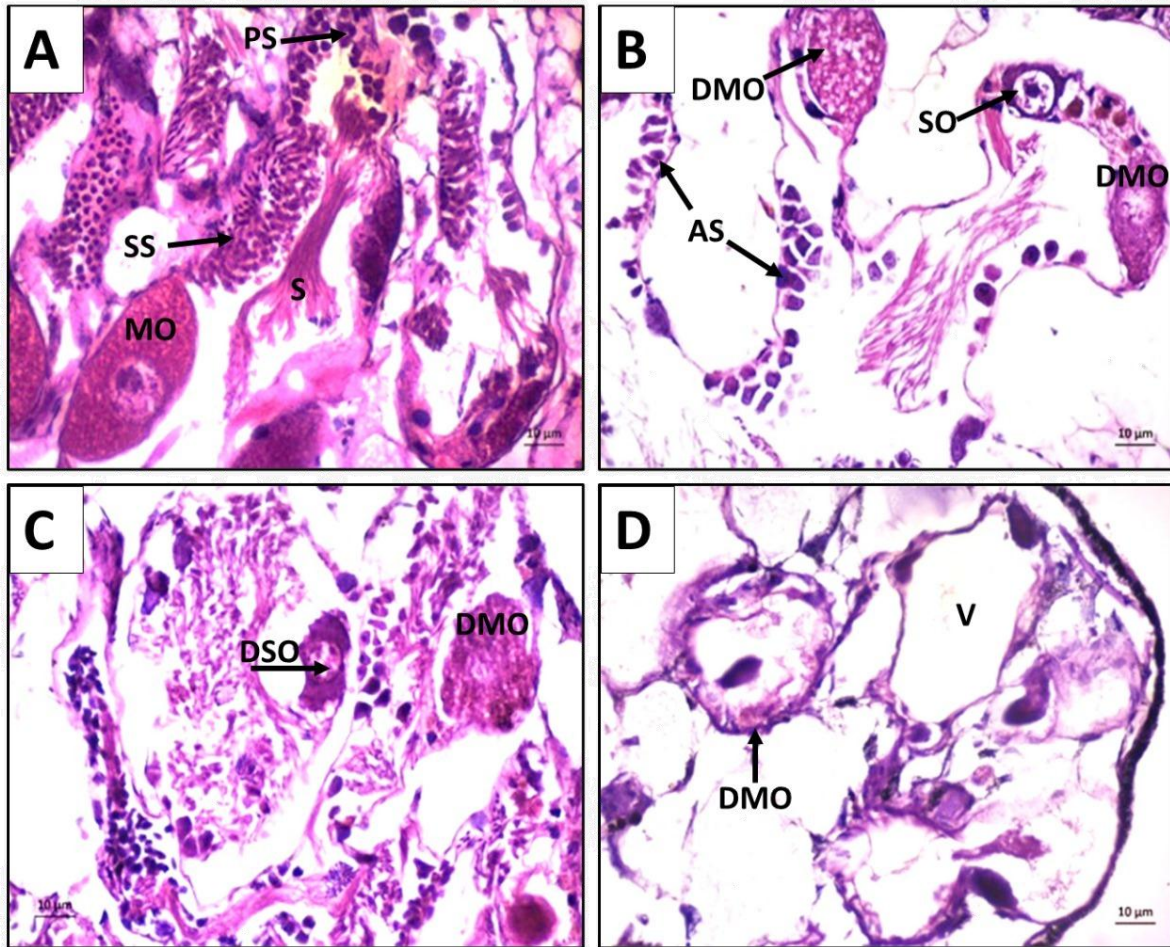


Fig. 6. Photomicrographs of the hermaphrodite gland of *Biomphalaria alexandrina* snails exposed to different water temperatures for 14 weeks. Snails exposed to A: room temperature, B: 30 °C, C: 32 °C, and D: 34 °C. The letters indicate PS; primary spermatocyte, SS; secondary spermatocytes, S: sperms, MO: Mature ovum; DMO: damaged mature ova; SO: secondary oocyte; AS: Atrophid spermatocytes; DSO: degenerated secondary oocyte; V: vacuoles.

2. Infection with *Schistosoma mansoni*:

Results of the present study in **Tables 1** and **2** summarized the impact of the different water temperatures on the infection of *B. alexandrina* snails by *S. mansoni*. The results revealed that increasing water temperature reduces the prepatent period of the infected snails. They were about 21, 19, and 18 days for the snails' groups at 25, 30, and 32 °C, respectively. There was a statistically significant difference (P -value <0.05) between the prepatent period of the infected snails maintained at room temperature (RT; 25°C) and those at 30 and 32 °C water temperature. In the same way, the shedding period was affected by temperature in which increased water temperature reduced the period of cercarial shedding. This period was approximately 17, 14, and 12 days for snails at 25, 30, and 32 °C, respectively. The mean

number of cercariae/snail for snails at 25 °C (111.1±14.39) was significantly higher ($P < 0.05$) than those at 30 and 32 °C (22.64±4.85 and 24.09±6.86, respectively). The results also indicate that the total number of produced cercariae was inhibited sharply by increasing the water temperature. The infected snails produced 2222, 634, and 265 cercariae at 25, 30, and 32 °C, respectively. However, at 34 °C, no cercariae were detected in the snails (**Table 1**). These results are in parallel with **Pflüger *et al.* (1984)**, who determined the effect of temperature on the length of the prepatent period in infected *B. glabrata* snails. They recorded a minimum prepatent period of 15 days among snails maintained at 32 and 33 °C. **Camargo *et al.* (2017)** also found that the higher the temperature, the shorter the period necessary for the development of the parasite and the higher the mortality of infected mollusks.

The results of the present study also showed that increasing water temperature reduced the survival rate of the infected snails. The infection rate of snails at 30°C (93.33%) was significantly higher compared with those at 25 and 32 °C (66.67% and 55%, respectively) (**Table 2**). This may be due to the double effect of exposure to both infection and high thermal stress, leading to the death of about one-third of the tested snails at 32 °C. Obviously, the number of infected snails was the lowest at 32 °C (11 snails) and the highest at 30°C (28 snails). However, the total cercariae production was higher at 25°C than at 30°C and 32°C. Meanwhile, the water temperature of 34 °C was high enough to prevent infection. These results indicate that the water temperature of 30 °C represents the most suitable temperature for inducing the highest infection rate of *B. alexandrina* snails by *S. mansoni*. This was previously reported by **Mangal *et al.* (2008)**, who stated that the mean infection burden in humans increases up to 30°C but then crashes at 35°C, primarily due to increased mortalities of the snail intermediate host according to a mechanistic model carried out to predict the impact of long-term temperature changes on the epidemiology of schistosomiasis.

Table (1): Effect of different water temperatures on the infected *Biomphalaria alexandrina* snails with *Schistosoma mansoni* miracidia

Temp. (°c)	No. of exposed snails	Survived snails at 1 st cercarial shedding	No. of infected snails	Prepatent period (days)	Shedding period (days)	Total no. of cercariae/snail	Total no. of cercarial production
RT (25)	30 (3×10)	30	20	20.64 ± 0.35 ^a	17.65 ± 0.86 ^a	111.1 ± 14.39 ^a	2222
30	30 (3×10)	30	28	18.17 ± 0.25 ^b	14.52 ± 1.14 ^a	22.64 ± 4.85 ^b	634
32	30 (3×10)	20	11	17.7 ± 0.19 ^b	12.45 ± 1.76 ^a	24.09 ± 6.86 ^b	265
34	30 (3×10)	NA	0	NA	NA	NA	NA

RT: Room Temperature

Means with different superscript letters indicate a significant difference at P-value <0.05

Table (2): Effect of different water temperatures on the survival and infection rates of infected *Biomphalaria alexandrina* snails with *Schistosoma mansoni* miracidia

Temperature (°c)	Survival rate (%)	Chi-square (χ^2) P-value	Infection rate (%)	Chi-square (χ^2) P-value
RT (25)	100		66.67	
30	100	$\chi^2 = 22.5$	93.33	$\chi^2 = 55.29$
32	66.67	P < 0.0001	55	P < 0.0001
34	NA		NA	

RT: Room Temperature

Means with different superscript letters indicate a significant difference at P-value <0.05

CONCLUSION

Based on the findings, it can be inferred that the potential rise in temperature resulting from global warming could adversely affect the population of *B. alexandrina* snails. These effects may include a decrease in survival rates and a reduction in reproductive output. Elevated temperatures in tropical regions may impact the transmission of the disease. In such circumstances, the intermediate host snail may shift its distribution to a different region that offers more favorable living conditions or will be unable to withstand the climatic changes in its current habitat.

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