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Effects of Different Salinities on Biology and Ultrastructure Topography of *Artemia salina*.

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ABSTRACT

This study evaluated the effects of salinity on *Artemia salina* from El Hamra Lake, Wadi El Natrun, Egypt. Different concentrations of salinities were tested; tap water (0.35), lake water (170) and hypersaline water (300) g/l against *A. salina* to assess survival, growth rate, morphometry and SEM topography of adults and hatchability of cysts for 7 days. Hypersaline and hyposaline concentrations caused 100 and 90 % reduction of survival rate, respectively and significant ($P \leq 0.03$) decrease of the body length. The hyposaline concentration increased significantly ($P \leq 0.05$) the width of thorax in males (2.7 ± 0.05) and females (2.6 ± 0.1) when compared to the control (2.1 ± 0.05 and 2.2 ± 0.05) mm, respectively. Both concentrations decreased the length of thorax and abdomen in males. At hyposaline and hypersaline groups, hatchability was reduced to 6 % and 0 %, respectively. The surface of branchiae, the maxillary gland and metepodites have more evaginations in the hypersaline concentration than control. Meanwhile, in the hyposaline media, it became nearly flattened. The hypersaline concentration caused reduction in eyes size, while the hyposaline increased it. In conclusion, *A. salina* exhibited different biological and morphological responses to regulate both hyposaline and hypersaline media.

Key words: *Artemia*, salinity, morphometry, ultrastructure, Wadi El-Natrun.

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INTRODUCTION

Salinity is an important environmental variable for estuarine organisms and its important physiological and ecological effects have been reviewed by Kinne, [1]. Surface waters can be classified according to their salt content as follows: freshwater < 0.5 g/l; oligohaline 0.5 : 4.0 g/l; mesohaline water 5 : 18 g/l; polyhaline water 18 : 30 g/l ; euhaline water 30 : 40 g/l and hyperhaline water > 40 g/l [2].

Artemia are among the few organisms that can adapt to very diverse salinities as low as 100 g/l and high as 240 g/l [3,4]. Laboratory experiments on New Zealand population of *A. franciscana* showed that the most favorable salinity for growth and survival rate to maturity was 100 – 170 g/l [5,6]. Under laboratory conditions, 11 geographical strains of *Artemia* had high survival rate at salinity 110 g/l [7]. Parthenogenetic population survived well at salinity 100 g/l while at 180 g/l, the survival was less than 50 % after 23 days of culture. Over 90 % of nauplii survived to maturity within this range, and individuals lived for \approx 5 months [8,9]. The effect of different salinities 35, 80, 120, 150 and 200 g/l was studied on the survival rate, growth rates and morphometric responses of four *Artemia* populations from northern Egypt. The bisexual population (Wadi El-Natron) exhibited its best survival at salinity 80 g/l [3]. Agh et al. [10] studied the effect of salinity ranged between 75 to 175g/l on the survival, growth rates and life span of three *Artemia* populations from Urmia Lake, Iran. They found that *Artemia* had significantly higher growth and survival at salinity ranged between 100-150 g/l and all parameters declined when salinity increased.

Janas et al. [11] studied the effects of salinity from 0.6 to 35 g/l on the survival and osmotic pressure of haemolymph in the indigenous prawn, *Palaemon adspersus*, and the non-indigenous prawn, *Palaemon elegans* from the Baltic Sea for 7 days. Survival rate was 80% - 100% in both species at salinity range from 1 to 35 g/l and declined in salinity above this value. Osmoregulatory capacities were considerable at salinities from 3 to 25 g/l. Babu and Shailender, [12] stated that the best percentage of hatching, high survival and development of the larval stages of the crustacean *Penaeus monodon* was recorded in the salinity ranged between 30 - 35 mg/l. However, when salinity increased to 40 mg/l, it reduced the same parameters.

Artemia has the ability to change its appearance under the influence of salinity [13,14]. The length and weight of late stages including adults of *Artemia monica* were reduced when salinity increased over 170 g/l [15]. Asem and Rastegar-Pouyani, [16] studied the biometry of *Artemia parthenogenetica* and *Artemia urmiana* nauplii after hatching of cysts in three different salinities (25, 35 and 45 ppt). *A. urmiana* showed the largest biometry of nauplii and meta-nauplii at concentration of 25 ppt and the smallest at 45 ppt. In *A. parthenogenetica*, the largest biometry of nauplii was obtained at 25 and 35 ppt and the smallest was at 45 ppt. Ben Naceur et al. [17] found highly negative significant correlations of salinity with some morphological characters of adult brine shrimp *Artemia salina* from 16 salt lakes in Tunisia.

One of the fascinating ecological adaptation mechanisms of the brine shrimp *Artemia* is its ability to survive in unstable environments by the production of dormant offspring. These encapsulated embryos can resist extreme environmental condition such as higher salinity, temperature and desiccation. However, when it needs to hatch, it must be hydrated and limited the degree of salinity [18]. The day of hatching, first brood production, and inter-brood duration, were increased as salinity was elevated [19]. Hatching in *A. monica* completely cesseded between salinities of 140 to 160 g/l due to inadequate cellular water for metabolic processes [20]. Aquatic organisms control the movement of water and ions across the exchange surfaces by altering the permeability of body surface and actively regulating the influx and efflux of water and ions [21]. Croghan [22] stated that *Artemia* died in distilled water after about 24 hrs due to a rapid loss of NaCl and gain of water. On contrary, *Artemia* in hypertonic medium, initiates active mechanisms for taking up NaCl and excreting excess water.

Scanning Electron Microscope (SEM) has become an important tool in the examination of both gross morphology and fine structures in many groups of crustaceans [23]. Many authors used SEM to study several structures in *Artemia* species and other crustaceans. Gilchrist, [24] indicated that the shell structure of *Artemia* has no surface pores. The new world species of *Artemia* have spherical frontal knobs and the old world species have conical ones [25]. The brine shrimp, *Artemia salina* has symmetrical surface structure of the mandibles [26].

This work was designed to study the impact of salinity as a critical abiotic factor on survival rate, morphometry, hatchability and alterations of surface topography of *Artemia salina* from Wadi El Natrun, Egypt.

MATERIALS AND METHODS

Experimental design

Artemia salina used in the present investigation was collected from El Hamra Lake, Wadi El Natrun, Egypt. Individuals were placed in plastic box containing Lake water. The animals were maintained under standard laboratory conditions, water temperature was 17 ± 2 °C and mild aeration was applied [3].

Adult *Artemia salina* (450 individuals) was used (50 individuals/replicate, one individual per 4 ml) according to El- Bermawi et al. [3]. *A. salina* individuals were acclimatized under laboratory conditions for one week before being used in the experimental study. Three concentrations of salinities with three replicates of each were used; 1st group was kept in Lake water (control, 170 g/l), 2nd group was kept in dechlorinated tap water (Hyposaline, 0.35 g/l) and 3rd group was kept in hypersaline water (300 g/l). The animals were fed on a mixed diet composed of dried yeast, wheat flour, soybean meal, squid and spirulina. Time intervals were 1, 3, and 7 days [27,3].

Determination of survival rate and body length of *Artemia salina*

Survival rate was determined as total number of alive *A. salina* during the period of experiment daily as a percentage related to those at the beginning of the experiment [28]. Body length was measured (mm) during sampling periods.

$$\text{Survival rate} = \frac{\text{Number of alive individuals}}{\text{Total number of individuals at the beginning of experiment}} \times 100$$

Morphometry of adult *Artemia salina*

Thirty individuals of each concentration/replicates were selected randomly. Samples were preserved in 70 % ethanol, flattened by placing them between slide and cover. Specimens were hydrated in a descending grade of ethanol then stained with Grnacher's Borax carmine. Specimens were differentiated in acidic ethyl alcohol (1 ml conc. HCL in one liter) and dehydrated in ascending series of ethyl alcohol. Specimens were cleared in clove oil and mounted in Canada balsam [29]. The following morphological parameters were measured; length of thorax and abdomen, width of thorax and the ratio of abdominal length/total length (expressed as percentage) using micrometer lens.

Hatchability of *Artemia salina* cycts

To study the effect of different concentrations of salinity on the cysts' hatchability, 90 cysts (30/concentration, 3 replicates, and 10/replicate) were collected and rinsed with distilled water. The experimental design of the effect of different salinities on cysts hatchability was done as the same as mentioned above on the adult *A. salina*. Hatched cysts were recorded daily over a period of 21 days [30].

Preparation of samples for Scanning Electron Microscope (SEM)

For SEM investigation, specimens of adult *A. salina* were prepared according to Haschemeyer and Mayers [31]. Specimens were cleaned by washing them gently several times in 70 % ethanol, and then fixed in 2 % gluteraldehyde buffered with phosphate pH 7.2. The specimens were then well dehydrated in a series of alcohol until 100 % and critical point dried. Then they were mounted onto metal stups and vaccumated for 8 min and then were coated with a thin layer of gold. The photographs in the magnification and scale bar desired were done by JEOL SEM, Faculty of Medicine, Tanta University, Egypt.

Statistical analysis

All data were analysed using Statgraphics (v5.1 software). Data were expressed as mean \pm SD. The statistical analysis was carried by unpaired One-way ANOVA to set the difference

between the control and treated groups of the experiment, setting the probability level to $P \leq 0.05$. Where ANOVA could not be applied, Kruskal Wallis test was used.

RESULTS

The results of the application of different concentrations of salinities on survival rate of *Artemia salina* for 7 days were recorded in Table (1). At 3rd day, the hypersaline and hyposaline concentrations caused 77 % and 70 % reduction of survival rate, respectively when compared to the control. At 7th day, the hypersaline and hyposaline concentrations caused 100 % and 90 % reduction, respectively. Hatchability at hyposaline group was reduced to 6 % and ceased to reach 0 % at hypersaline group when compared to the control, which was 100 % hatchability.

Concerning the body length, the hyposaline concentration caused significant decrease of the body length in all time points of the experiment (ANOVA, $P \leq 0.03$). The length of body was 5.1 ± 0.1 mm at 7th day when compared to the control (7.2 ± 0.05 mm). The hypersaline concentration caused significant decrease in all time points of the experiment (ANOVA, $P \leq 0.003$). The length of body was 4.3 ± 0.1 mm at 7th day when compared to control (7.2 ± 0.05 mm). For comparison, the decrease of body length of *A. salina* was greater at the hypersaline than the hyposaline concentration (Table 1).

Table 1: Effect of different concentrations of salinities on the survival rate and body length of *Artemia salina* after 7 days of exposure

Exposure period	Salinities	Control* (170 g/l)	Hyposaline (0.35 g/l)	Hypersaline (300 g/l)
	Parameters			
1 day	Survival rate %	100	56	43
	Total length(mm)	6.6 ± 0.4	$5.8 \pm 0.1\#$	$5 \pm 0.1\#$
3 days	Survival rate %	93	30	23
	Total length(mm)	6.7 ± 0.2	$5.2 \pm 0.1\#$	$4.7 \pm 0.1\#$
7 days	Survival rate %	83	10	0
	Total length(mm)	7.2 ± 0.05	$5.1 \pm 0.1\#$	$4.3 \pm 0.1\#$

Note; $n = 10$ individuals, data were expressed as means \pm SD, # indicates significant difference when $P \leq 0.05$ (ANOVA). * Lake water.

Data shown in Table (2) indicated that width of thorax in males' insignificantly increased (Kruskal Wallis, $P > 0.05$) in hyposaline concentration (2.7 ± 0.05 mm). However, it was unaffected in hypersaline concentration (2.1 ± 0.1 mm) when compared to the control (2.1 ± 0.05 mm) at 1st day. At 3rd day, it insignificantly decreased (2.5 ± 0.05 mm) at hyposaline concentration and significantly decreased (2.06 ± 0.1 mm, ANOVA, $P = 0.001$) at hypersaline concentration when compared to the control males (2.8 ± 0.1 mm). At 7th day, the width of thorax unaffected in hyposaline concentration and insignificantly (Kruskal Wallis, $P > 0.05$) decreased in hypersaline concentration when compared to the control. It was 2 ± 0.05 and 1.8 ± 0.1 mm in hyposaline and hypersaline concentrations, respectively, when the control group was 2 ± 0.1 mm.

The length of thorax showed insignificant decrease (3 ± 0.1 mm) at the hyposaline concentration and significant decrease (2.4 ± 0.1 mm, ANOVA, $P = 0.0005$) in hypersaline concentration at 1st day when compared to the control (3.3 ± 0.15 mm). At the 3rd day, it showed insignificant decrease (3.1 ± 0.1 mm) at the hyposaline concentration and significant decrease (2.2 ± 0.1 mm, ANOVA, $P = 0.0003$) at the hypersaline concentration when compared to the control group (3.5 ± 0.05 mm). At the 7th day, it showed significant decrease (Kruskal Wallis, $P \leq 0.00001$) at both hyposaline and hypersaline concentrations. It recorded 2.7 ± 0.05 and 2 ± 0.1 mm in the hyposaline and hypersaline concentrations, respectively, while the control was 3.8 ± 0.05 mm (Table 2).

Table 2: Effect of different concentrations of salinities on the morphometry of the male *Artemia salina* after 7 days

Exposure period	Salinities	Control* (170 g/l)	Hyposaline (0.35 g/l)	Hypersaline (300 g/l)
	Morphometric parameters			
1 day	Width of thorax (mm)	2.1 ± 0.05	2.7 ± 0.05	2.1 ± 0.1
	Length of thorax (mm)	3.3 ± 0.15	3 ± 0.1	$2.4 \pm 0.1\#$
	Length of abdomen (mm)	3 ± 0	$2.5 \pm 0.05\#$	2.1 ± 0.05
	Abdominal length / total length (%)	45	43	42
3 days	Width of thorax (mm)	2.8 ± 0.1	2.5 ± 0.05	$2.06 \pm 0.1\#$
	Length of thorax (mm)	3.5 ± 0.05	3.1 ± 0.1	$2.2 \pm 0.1\#$
	Length of abdomen (mm)	3.1 ± 0.1	2 ± 0.05	$2 \pm 0.05\#$
	Abdominal length / total length (%)	46	36	42
7 days	Width of thorax (mm)	2 ± 0.1	2 ± 0.05	1.8 ± 0.1
	Length of thorax (mm)	3.8 ± 0.05	$2.7 \pm 0.05\#$	$2 \pm 0.1\#$
	Length of abdomen (mm)	3.1 ± 0.05	$2 \pm 0.1\#$	$1.9 \pm 0.05\#$
	Abdominal length / total length (%)	43	39	44

Note; $n = 10$ individuals, data were expressed as means \pm SD, # indicates significant difference when $P \leq 0.05$ (ANOVA / Kruskal Wallis). * Lake water.

The length of abdomen showed significant decrease in hyposaline concentration at the 1st and the 7th day (ANOVA, $P = 0.001$), insignificant decrease at 3rd day. It was 2.5 ± 0.05 mm at 1st day, 2 ± 0.05 mm at the 3rd day and 2 ± 0.1 mm at the 7th day when compared to control values 3 ± 0.0 , 3.1 ± 0.1 and 3.1 ± 0.05 mm at 1, 3 and 7 days, respectively. In hypersaline, it showed insignificant decrease at the 1st day, significant decrease at the 3rd and the 7th day (Kruskal Wallis/ANOVA, $P \leq 0.0001$). It was 2.1 ± 0.05 mm at the 1st day, 2 ± 0.05 mm at the 3rd day and 1.9 ± 0.05 mm at the 7th day when compared to the control values 3 ± 0 , 3.1 ± 0.1 and 3.1 ± 0.05 mm at 1, 3 and 7 days, respectively (Table 2).

Ratio of abdominal length/total length decreased in hyposaline and hypersaline concentrations at the 1st (43 %) and the 3rd (42 %) days when compared to the control group (45 %, Table 2). At the 3rd day, it recorded 36 % and 42 % reduction in hyposaline and hypersaline concentrations, respectively, when compared to the control, 46 %. At the 7th day, the ratio was 44 % at the hypersaline concentration when the control was 43%.

Data in Table (3) indicated that, the width of thorax in female significantly increased in hyposaline and hypersaline concentrations (ANOVA, $P \leq 0.001$) when compared to the control at the 1st day. It was 2.6 ± 0.1 mm in the hyposaline and 2.4 ± 0.05 in hypersaline concentrations while the control was 2.2 ± 0.05 mm. At the 3rd day, it insignificantly decreased at hyposaline (2.4 ± 0.1 mm) and hypersaline (2.3 ± 0.05 mm) concentrations when compared to the control (2.9 ± 0.1 mm). At the 7th day, the width of thorax significantly increased in hyposaline concentration (2.3 ± 0.05 mm, ANOVA, $P = 0.003$) and significantly decreased (2 ± 0.05 mm, ANOVA, $P = 0.01$) in hypersaline concentration when compared to control (2.1 ± 0.1 mm).

Table 3: Effect of different concentrations of salinities on the morphometry of the female *Artemia salina* after 7 days

Exposure period	Salinities			
	Morphometric parameters	Control* (170 g/l)	Hyposaline (0.35 g/l)	Hypersaline (300 g/l)
1 day	Width of thorax (mm)	2.2 ± 0.05	$2.6 \pm 0.1\#$	$2.4 \pm .05\#$
	Length of thorax (mm)	3.3 ± 0.05	2.4 ± 0.05	$2.5 \pm .05\#$
	Length of abdomen (mm)	3.1 ± 0.1	$2.1 \pm 0.1\#$	$2.1 \pm 0.1\#$
	Abdominal length / total length (%)	46	36	42
3 days	Width of thorax (mm)	2.9 ± 0.1	2.4 ± 0.1	2.3 ± 0.05
	Length of thorax (mm)	3 ± 0.05	3 ± 0.05	2.7 ± 0.05
	Length of abdomen (mm)	2.5 ± 0.1	2 ± 0.1	2 ± 0.05
	Abdominal length / total length (%)	37	38	42
7 days	Width of thorax (mm)	2.1 ± 0.1	$2.3 \pm 0.05\#$	$2 \pm 0.05\#$
	Length of thorax (mm)	3 ± 0.05	3 ± 0.1	2.9 ± 0.1
	Length of abdomen (mm)	3.1 ± 0.1	$2.5 \pm 0.05\#$	$2.3 \pm .05\#$
	Abdominal length / total length (%)	43	46	53

Note; $n = 10$ individuals, data were expressed as means \pm SD, # indicates significant difference when $P \leq 0.05$ (ANOVA/ Kruskal Wallis). * Lake water.

The mean length of thorax showed insignificant decrease in the hyposaline group and significant decrease (ANOVA, $P = 0.001$) in the hypersaline group at the 1st day when compared to the control group. It was 2.4 ± 0.05 , 2.5 ± 0.05 and 3.3 ± 0.05 mm at the hyposaline, hypersaline concentrations' and the control, respectively. At the 3rd day, it is unaffected at the hyposaline group and insignificantly decreased at the hypersaline group when compared to the control group. The mean values were 3 ± 0.05 and 2.7 ± 0.05 mm in the hyposaline and hypersaline concentrations, respectively, while the control was 3 ± 0.05 mm. At the 7th, it is unaffected at the hyposaline group (3 ± 0.1 mm) and insignificantly decreased at the hypersaline group (2.9 ± 0.1 mm), but the control was 3 ± 0.05 mm (Table 3).

The length of abdomen showed significant decrease in hyposaline concentration at the 1st day (ANOVA, $P = 0.007$), the 7th day (ANOVA, $P = 0.003$) and insignificantly decreased at the 3rd day. It recorded 2.1 ± 0.1 mm at the 1st day, 2 ± 0.1 mm at the 3rd day and 2.5 ± 0.05 mm at

the 7th day when compared to the control values 3.1 ± 0.1 , 2.5 ± 0.1 and 3.1 ± 0.1 mm, respectively (Table 3).

The ratio of abdominal length/total length decreased in hyposaline (36 %) and hypersaline (42 %) concentrations compared to the control group (46 %) at 1st day. At the 3rd and the 7th days, it showed increase in hyposaline and hypersaline concentrations and recorded 38, 42, 46 and 53 % in hyposaline and hypersaline concentrations at the 3rd and the 7th days, when the control group was 37 and 43 %, respectively (Table 3).

Adult *A. salina* exhibited different morphological alterations to regulate both hyposaline and hypersaline media (Figs. 1, 2 and 3). The surface of branchiae, the maxillary gland and metepodites became more evaginated in the hypersaline concentration. Meanwhile in the hyposaline media, it became nearly flattened when compared to the normal control individuals. Generally, the body of *A. salina* looked more fragile in the hyposaline concentration. Observations of the size of eye showed difference in both concentrations in comparison to the eye size of control one. The hypersaline concentration caused reduction in the size of the eyes while the hyposaline one increased their sizes (Fig. 4).

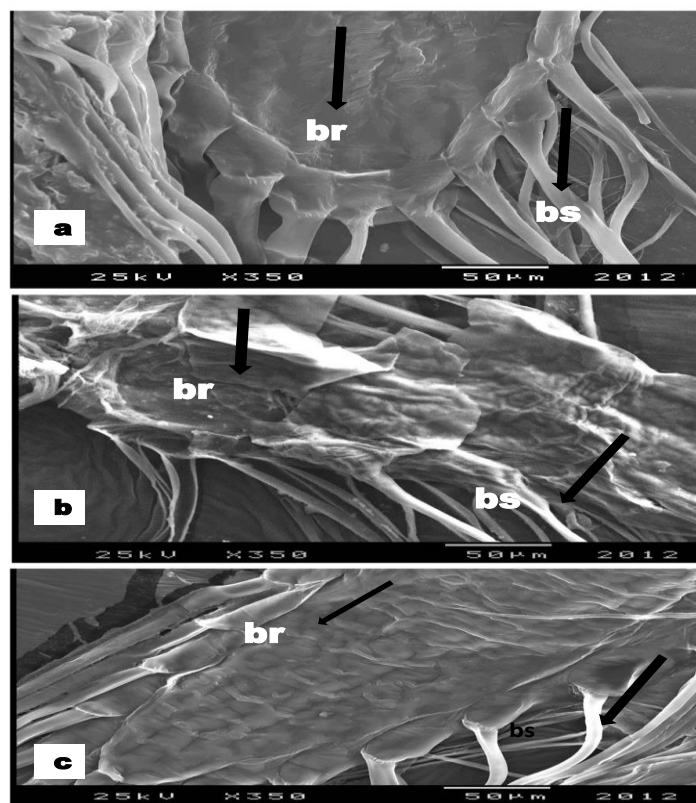


Fig (1): Scanning electron micrographs showing surface view of the branchiae of *A. salina* exposed to different concentrations of salinities (a) *A. salina* (control, 170 g/l) showing normal structure of the surface; (b) *A. salina* exposed to hyposaline concentration (0.35 g/l, nearly flattened, arrows) and (c) *A. salina* exposed to hypersaline concentration (300 g/l, with evaginations, arrows). br, branchiae; bs, branchial cetae. Scale bar, 50 μ m.

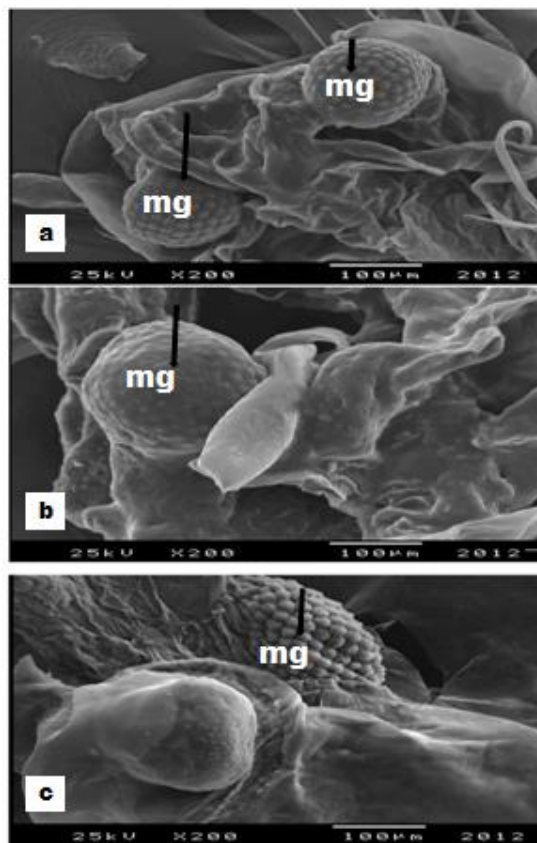


Fig (2): Scanning electron micrographs showing external surface of the maxillary gland of *A. salina* exposed to different concentrations of salinities (a) *A. salina* (control, 170 g/l) showing normal structure of the surface; (b) *A. salina* exposed to hyposaline concentration (0.35 g/l, flattened, arrow) and (c) *A. salina* exposed to hypersaline concentration, (300 g/l, with evaginations, arrow). mg, maxillary gland. Scale bar, 100 µm.

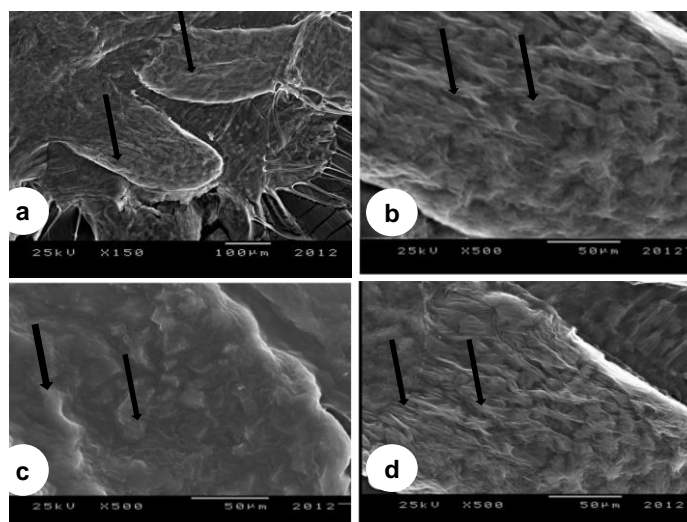


Fig (3): Scanning electron micrographs showing the metepodite of *A. salina* exposed to different concentrations of salinities (a) *A. salina*, (Control, 170 g/l), (arrows), Scale bar, 100 µm; (b) *A. salina*, (Control, magnified); (c) *A. salina* exposed to hyposaline concentration, (0.35 g/l, nearly flattened, arrows) and (d): *A. salina* exposed to hypersaline concentration, (300 g/l, with evaginations, arrows). Scale bar, 50 µm.

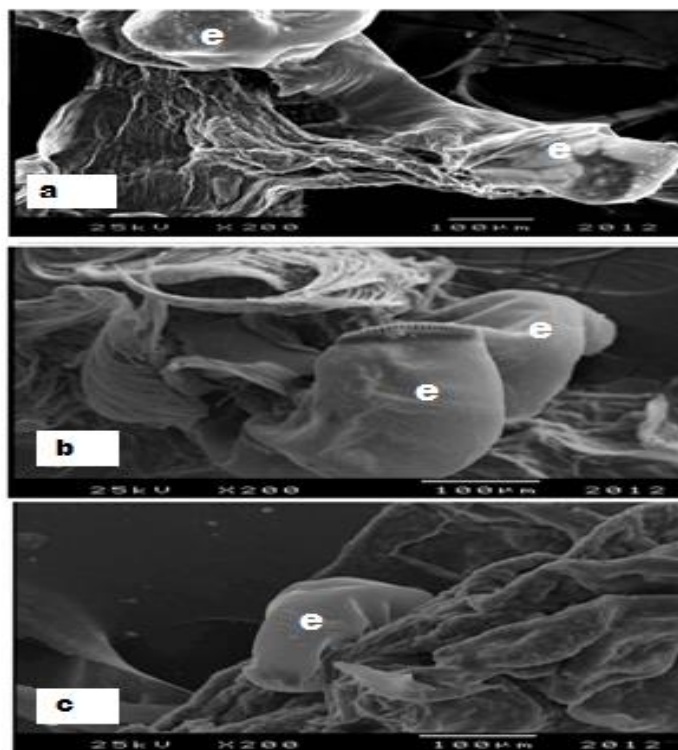


Fig (4): Scanning electron micrographs showing the eyes of *A. salina* subjected to different concentrations of salinities (a) *A. salina* (control, 170 g/l); (b) *A. salina* exposed to hyposaline concentration, (0.35 g/l, swollen) and (c) *A. salina* exposed to hypersaline concentration, (300 g/l, shrunk). e, eye. Scale bar, 100 µm.

DISCUSSION

The achieved results indicated that both hypersaline and hyposaline concentrations caused reduction of survival rate at the 3rd and the 7th day. These results are in agreement with Wear and Haslett [8] who found that the bisexual *A. franciscana* population showed higher mortalities at salinities under 60 g/l and over 120 g/l. 90 % survival was recorded at salinity 190 g/l and 0% survival rate was at salinity 230 g/l of *A. monica* from Mono lake, California, USA [32]. One hundred percent mortality of *Artemia urmiana* population was observed at salinity over 250 g/l from Urmia Lake, Iran [33,10]. The decline in survival rates of adult *A. monica* with increasing salinity may be related to the failure in its biochemical and osmoregulatory processes [34,9,35]. Oxygen content of marine Lake water decreases from 6 to 4 mg/l when salinity increased over 168 g/l [34]. Low oxygen concentrations (Hypoxia) present at salinity above 200 g/l. This Hypoxia can be an additional stressor contributing to mortality of *Artemia* because it reduce the respiratory rate which leading to a breakdown in osmoregulation and subsequently increase the mortality [36, 37]. Protein synthesis and ATP levels in naupliar shrimp decreased when salinity increased because the shrimp partitioned the available energy between ion transport and protein synthesis in response to high salinity. Thus, energy dependent processes such as growth and reproduction are likely to be reduced at high salinities, as more energy is required for the maintenance of osmotic homeostasis [38].

The present results indicated that total length of *A. salina* decreased gradually in the hyposaline and hypersaline concentrations but the reduction was greater in hypersaline. These data are agreement with Agh et al. [10]; Soundarapandian and Saravanakumar, [39] who reported that growth rate was inversely proportional to salinity. Increase of salinity results in reduction of brine shrimp body size especially the abdomen. In Wadi El Natrun strain of *A. salina*, the highest growth performance was at the salinity range between 120 and 260 g/l [3]. *Artemia franciscana* showed the best growth rates at 100 - 120 g/l [4].

The current results indicated that the width of thorax increased in hyposaline and decreased in hypersaline concentration. However, the length of thorax and abdomen as well as ratio of abdominal length/total length decreased in hyposaline and hypersaline at all time points. It was established that *Artemia* has the ability to change its appearance under the influence of salinity in previous works by Gilchrist, [40]; Rodríguez-Almaraz et al. [13] and Ben Naceur et al. [41]. A morphometric analysis of 25 Siberian populations of *Artemia* demonstrated that body length and abdomen / body length ratio were the most stable but inversely proportional with high salinity [42]. Biometry of nauplii of *A. urmiana* showed statistical differences when their cyst hatched in different salinities, the body size was 2.8 mm at salinity 125 g/l and it was 2.2 mm at salinity 145 g/l [43]. There were highly significant negative correlations between high salinity and the width of the 3rd abdominal segment, width of the head, diameter of the compound eyes of *Artemia* in Tunisia [17].

The recorded results indicated a reduction/cessation of *Artemia salina* cysts hatchability according to salinity concentrations. Reduction in the hatching of *Artemia salina* cysts was observed as salinity increased as nauplii partitioned the available energy between ion transport and protein synthesis. At high salinities, more energy is required for the maintenance of osmotic homeostasis [19] or it can be attributed to shifts in cyst carbohydrate metabolism [20].

The present work observations recorded surface alterations of *A. salina* under the effect of different salinities. The surface of the maxillary gland, metepodites and branchiae became more evaginated in the high salinity. High salinity considered as hyperosmotic medium in relation to *Artemia* body fluids. So, *Artemia* showed some alterations in the morphology of these organs to increase the absorption surface area. Croghan [22] found that aquatic organisms control the movement of water and ions across the exchange surfaces by altering the permeability of body surface and actively regulating the influx and efflux of water and ions. There are different ways in adult *A. salina* for osmotic and ionic regulation; in the concentrated media, they drink to replace water lost by osmosis to the hyperosmotic external medium. There were eleventh pairs of the branchiae in *Artemia*, the epithelium of the first ten pairs of these branchiae is capable of actively excreting NaCl from the haemolymph into a hypertonic medium [22]. The metepodites of *Artemia* appear to be the sites of outward ion transport. The maxillary gland had very high Na/K⁺-ATPase enzyme specific activity that increase in proportion to the salinity of the external medium [19]. Thickened metepodite epithelium in brine shrimps is another way of adaptation to concentrated medium. The increased thickness of the metepodites is due to the multiplication of dark cells in the metepodite epithelium in response to transfer of the brine shrimp to high salinity media [44,45,46,47].

In the present work, surface of the maxillary gland, metepodites and branchiae became flattened in the hyposaline group. So, *Artemia* showed some alterations in the morphology of these organs to increase the uptake of water and lose salts to the external medium through their surface area. Croghan, [22] recorded mortality of *Artemia* in distilled water after about 24 hrs due to a rapid loss of NaCl and gain of water. Copeland, [48] suggested that metepipodites showed areas of high chloride permeability in the cuticle of *A. salina* by using the silver staining method. The hypoosmotic regulation in both adults and embryos of Cladocera, determined mainly by excretion of salts in special epipodite cells, metepodites and in cells of the nuchal (neck) organ [49,50, 51].

CONCLUSION

It can be concluded that optimum salinity is as ecological requirement for *Artemia salina* and it responded differently by biological and morphological changes for both hyposaline and hypersaline stresses.

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