



## EFFECTS OF DESALINATION BRINE ON THE FECUNDITY OF BRINE SHRIMP *ARTEMIA FRANCISCANA* FED ON RICE BRAN

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### Abstract

Brine water drained from the desalination stations represents environmental concerns because of its extremely high salinity. *Artemia* (brine shrimp) is one of the crustaceans that can live in increased saline water. So, it can live in the desalination brine water. This study investigated the possibility of growing *Artemia* (*Artemia franciscana*), an essential live food for the aquaculture industry, in the brine water disposed of during the desalination process. Nine reproductive characteristics were examined for *Artemia* growing in desalination brine water, compared to seawater. Both types of water were brought from the Rumaila water desalination facility on the Mediterranean Sea in Marsa Matrouh, Egypt. The experiment included brine water of salinities: 50, 60, 70, and 80 ppt and two seawater salinities: 38 and 50 ppt. The food source used was the rice bran suspension to feed *Artemia* during the experiment. The results illustrated that the pre-reproductive and reproductive periods and % offspring encysted had higher values in higher salinities of brine water than seawater. The rate of offspring encysted in the brine water of 70 ppt reached 72.42%, followed by the brine water of 60 ppt. The results showed that the *Artemia* could grow in the brine water of the desalination plants till the adult stage. The results show no significant difference between both types of water in some variables.

**Key words:** brine shrimp, rejected brine, seawater desalination, reproduction, live food

*Artemia* (brine shrimp) is an euryhaline crustacean that can live in hypersaline lakes and ponds (Henry et al., 2019; Lenz and Browne, 1991). The use of brine shrimp *Artemia* as a diet for the larval culture of many species became commonly applied with the evolution of hatchery aquaculture. It was chosen for its ease of use and nutritional content (Conceição et al., 2010). *Artemia* cysts (eggs) may be kept in packs for a long time and then utilized as an off-the-shelf meal. It only takes 24 hours to incubate, making them the most practical and labor-intensive live food to cultivate larval fish and shrimp species (Conceição et al., 2010). *Artemia* was also an excellent food source among all available species because it can be easily digested and accepted by the fish larvae (Hoa, 2002; Khoi, 2006).

*Artemia* cysts, which come from a small range of inland salt lakes, are exported worldwide (Van Stappen et al., 2020). *Artemia* production in evaporation ponds could be a credible option to deal with potential cyst deficiencies and high pricing in recent years, especially in areas with high volumes of brine water from desalination processes. Evaporation ponds are a kind of brine disposal that consists of small, bordered earthen basins where brine is gently evaporated using direct sun radia-

tion (Panagopoulos et al., 2019). Evaporation basins are used to condense and decrease the volume of incoming discharge by evaporating (Sharkh et al., 2022). Unconventional waters, such as desalinated waters (Sharifinia et al., 2022) are very important for aquaculture (Soleimani-Sardo and Khanjani, 2023). Brine water from the desalination plants with high salinities is suitable for growing *Artemia* (Ahmed et al., 2004; Sorgeloos, 1980). Brine water is used to enhance the aquaculture industry by producing *Artemia*, the most widely used live food in finfish and crustacean aquaculture (Kandathil Radhakrishnan et al., 2020). This paper studies the ability to grow *Artemia* in brine water, which is drained from the desalination stations.

Plant ingredients, such as rice bran, soybean meals, and others, can improve the biological traits of *Artemia* (Balachandar and Rajaram, 2019). *Artemia* requires carbohydrates to grow to the adult stage; rice bran showed its carbohydrate provision to *Artemia* and showed higher percentages than other diets (Balachandar and Rajaram, 2019). Rice bran is a valuable by-product of the rice milling industry, with high generated amount yearly (Sohail et al., 2017; Yu et al., 2021). Rice bran is a cheap cereal by-product (Devi et al., 2021). It can be a functional and

nutritious supplement (Devi et al., 2021). It is also used as one of the carbohydrate fillers in some healthy foods, where it is an inexpensive filler (Sujarwanta et al., 2021). Sorgeloos et al. (1980) and Vanhaecke and Sorgeloos (1989) studied the effect of rice bran in comparison to microalgae *Dunaliella* on the length of *Artemia* within seven days; the results showed that the rice bran gave higher results. Balachandar and Rajaram (2019) also examined rice bran feed separately and in combination with *Tetraselmis* sp. and *Chaetoceros* sp. to study its effect on *Artemia franciscana*. It gave the highest growth rates compared to other discrete feeds of *Chaetoceros* sp., *Nannochloropsis* sp., *Tetraselmis* sp., *Thalassiosira* sp., *Isochrysis* sp., and soybean meal. One of the challenges in *Artemia* culture is increasing the inorganic nitrogen resulting from *Artemia* waste. These nitrogen forms of ammonia and ammonium may impair the growth and survival of cultured *Artemia* (Khanjani et al., 2022 a; Serra et al., 2015). Rice bran, one of the carbon sources, can balance carbon and nitrogen with its high carbohydrate content (Yu et al., 2021). It metabolizes ammonia and enhances water properties (Khanjani and Sharifinia, 2022 b; Robles-Porchas et al., 2020). It encourages the formation of a heterotrophic microbial population, converts ammonia and solid waste into biofloc, which *Artemia* can easily consume as food, and improves nutrient cycling (Khanjani et al., 2022 b, c; Khanjani and Sharifinia, 2022 a).

This research investigates the ability of brine water discarded from a desalination plant to grow *Artemia* till the adult stage and their effect on the reproductive characteristics of *Artemia* by feeding them rice bran as a natural feed. The brine water's (50, 60, 70, and 80 parts per thousand (ppt)) effect was compared to the impact of seawater (of 38 and 50 ppt).

## Material and methods

### Water cultures

Various seawater and brine water concentrations were prepared by boiling seawater and brine water in glass tanks with an electrical hot plate until the required salinity was obtained. The salt concentration was then measured using a calibrated EC meter (Thermo Scientific Orion Star A329, USA) (Assiry et al., 2010). Solutions for different salinities were as follows: seawater (38 and 50 ppt) and brine water (50, 60, 70, and 80 ppt). The seawater and brine water were brought from the Rumaila water desalination facility in Marsa Matrouh on the Mediter-

anean Sea, Egypt. Water analyses of both water types are presented in Table 1. Every seven days, about 20% of each water salinity was substituted by new water with the same salinity (Browne and Wanigasekera, 2000).

### Culture conditions

The brine shrimp *Artemia franciscana* cysts (EG grade, INVE® Aquaculture, Belgium) were hatched in natural seawater under the ideal conditions in the laboratory (Sorgeloos, 1986). Rice bran suspension was used to feed the fresh nauplii during a 24-hour acclimatization phase (Triantaphyllidis et al., 1995).

The rice bran suspension was made by dissolving stabilized rice bran (Oryza®, Egypt) in seawater (Balachandar and Rajaram, 2019; Dhert et al., 1992). Where 20 g of rice bran powder has omega 3 (63.2 mg), omega 6 (1420 mg), vitamin B<sub>5</sub> (14.8% RDA), vitamin B<sub>6</sub> (40.8% RDA), vitamin E (5% RDA), iron (20.6% RDA), zinc (8% RDA), and manganese (100% RDA). The rice bran suspension should have been sieved to be easily digested by *Artemia*, in the range of 1–50 µm (Anh et al., 2009; Dhont and Van Stappen, 2003; Punitha et al., 2007), 60 µm (Dhert et al., 1992), or 80 µm (Platon, 1985).

After the adaptation phase of 24 h, individuals were placed directly in transparent plastic tanks of different treatments (seawater of 38 and 50 ppt and brine water of 50, 60, 70, and 80 ppt concentrations). When *Artemia* males began to clasp females, we moved five maturated *Artemia* pairs to 5 transparent plastic jars per water treatment (each jar holds 250 mL). After one of the partners died, males and females were mated with a survivor from another pair developed at the same salinity (Browne and Wanigasekera, 2000).

The jars were placed in a constant temperature refrigerator, where the temperature was 28±1°C (Dhont and Van Stappen, 2003; Medina et al., 2007; Norouzitallab et al., 2014) and under steady fluorescent lighting (Browne and Wanigasekera, 2000). Jars into each treatment were randomly relocated every three days to reduce differential impacts (Browne and Wanigasekera, 2000). There was no artificial aeration utilized as the oxygen concentration was 2.5–5 mg/L (Cisneros and Vinatea, 2009; Dhont and Van Stappen, 2003). The dissolved oxygen was measured by a multi-parameter analyzer (Thermo Scientific Orion Star A329, USA). The pH of the various water salinities is kept in the range of 6.5–8 by adding a few drops of buffer solutions of NaHCO<sub>3</sub> or HCl using a pH meter (Thermo Scientific Orion Star A329, USA) (Dhont and Van Stappen, 2003).

Table 1. Water analyses of seawater and desalination brine water from Rumaila water desalination station, the Mediterranean Sea in Marsa Matrouh, Egypt

Water type	pH	EC dS/m	Soluble salts (mg/L)=ppm												SAR	TDS ppm
			Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>	Fe	Zn	Mn	Cu		
Seawater	8.45	60.10	50.00	109.20	10959.50	440.70	0.00	0.00	16685.00	1411.20	0.11	0.02	0.05	0.02	197.9	38080
Brine water	7.15	63.20	740.00	1332.00	12535.00	491.40	0.00	1220.00	23927.00	556.80	0.03	0.01	0.02	0.01	63.4	50560

### Lifespan and reproductive experiments

The jars were tested for offspring production daily. *Artemia* was counted and checked under a binocular microscope (Leica EZ4, Leica Microsystems, Germany). The experiment lasted for 38 days. Individuals were followed throughout their lives, and nine fecundity characteristics were documented for every treatment: (1) Female pre-reproductive phase (days); (2) Female reproductive period (days); (3) Offspring per brood; (4) Broods per female; (5) Offspring per female; (6) Inter-brood interval (days); (7) Percentage of offspring encysted (%); (8) Reproductive females' lifespan (days); and (9) Male lifespan (days) (Browne and Wanigasekera, 2000; Triantaphyllidis et al., 1995).

### Statistical analyses

The figures of the nine reproductive characteristics of *Artemia* were collected from the experiment. They were evaluated with statistical software (IBM SPSS-22 One-way ANOVA, USA) for Windows. The analysis followed a regular procedure, with all of the variables measured being input into the program simultaneously. The multiple comparison (Tukey HSD) test was applied to identify the differences and supplement the post-hoc analysis. The mean standard error ( $\pm$ ) was given with all data.

## Results

### Female pre-reproductive period

Figure 1 illustrates the effect of various seawater and desalination brine water salinities on the pre-reproductive period of *Artemia* when they are fed on rice bran. All *Artemia* cultivated in higher salinities showed a more extended pre-reproductive period with increasing salinities. This increase started from the brine water of 60 ppt. The pre-reproductive period was in the range of 23.80–25.20 days. However, lower salinities of 38 and 50 ppt had shorter pre-reproductive periods of 18.20–19.60 days. There was no substantial difference among the low salinities (Tukey,  $P > 0.05$ ).

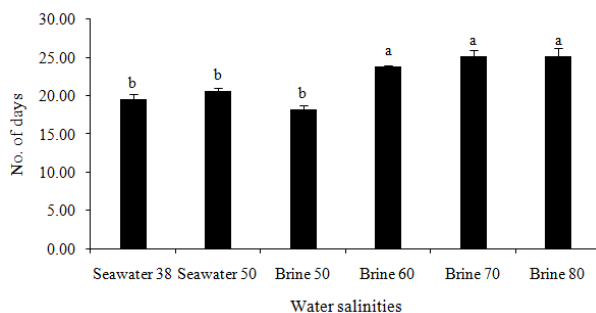


Figure 1. The effect of various water salinities (ppt) on the female pre-reproductive period (days), *Artemia franciscana* was fed on rice bran. Data expressed as mean  $\pm$  SE. Error bars represent the standard error of the mean. Bar columns having different letters are significantly different at  $P < 0.05$  (Tukey test)

### Female reproductive period

For the higher brine water salinities, the female reproductive period was longer than in the lower salinities of 38 and 50 ppt (see Figure 2). It is obvious that the reproductive period was decreasing in higher salinities, starting from brine water (60 ppt). However, the results did not show significant differences amongst each group of the various salinities.

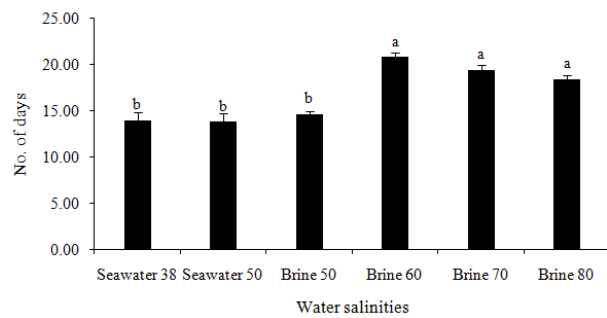


Figure 2. The impact of various water salinities (ppt) on *Artemia franciscana*'s female reproductive period (days), where rice bran was used as food. Data presented as mean  $\pm$  SE. Error bars represent the standard error of the mean. Bar columns having different letters are significantly different at  $P < 0.05$  (Tukey test)

### Offspring per brood

The number of offspring per brood of *Artemia* individuals fed on rice bran did not show significant differences (Tukey,  $P > 0.05$ ) among all water salinities, as shown in Figure 3. However, the brine water 50 ppt had the maximum number of offspring per brood, about 92. At the same time, the lowest value of offspring per brood happened in the brine water of 80 ppt, 53 offspring. It does not appear that the offspring per brood is a major salinity factor.

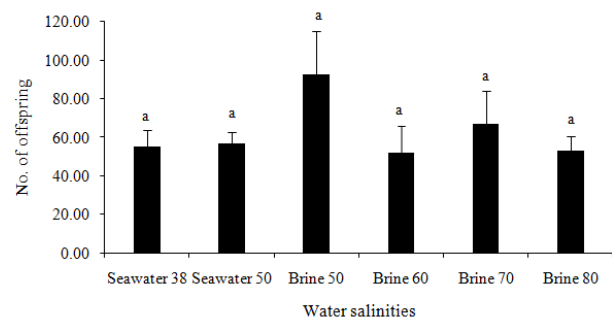


Figure 3. The number of offspring per brood of *Artemia franciscana* individuals reared in six salinities, fed with rice bran. Data expressed as mean  $\pm$  SE. Error bars represent the standard error of the mean. Bar columns having different letters are significantly different at  $P < 0.05$  (Tukey test)

### Broods per female

According to the number of broods per female, there was a variation among the individuals cultivated in the different water salinities, as illustrated in Figure 4. Where the female animals were grown in seawater 50 ppt, brine water 50 and 80 ppt showed a large number of broods of

3. Even though the *Artemia* females cultivated in brine water 70 ppt exhibited the lowest number of broods, the mean value was 1.60.

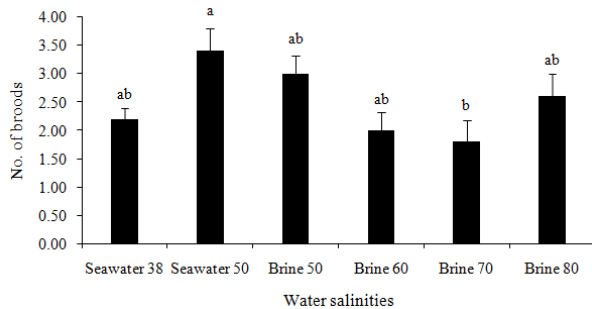


Figure 4. The number of broods per female reared in the various water salinities using rice bran feed. Data represented as mean ± SE. Error bars represent the standard error of the mean. Bar columns having different letters are significantly different at P<0.05 (Tukey test)

**Offspring per female**

Results did not show significant differences for offspring per female, except for the brine water of 50 ppt. *Artemia* individuals reared in brine water 50 ppt had the maximum production of offspring of 334. All *Artemia* populations had the average number of offspring between 122 and 190, as reflected in Figure 5.

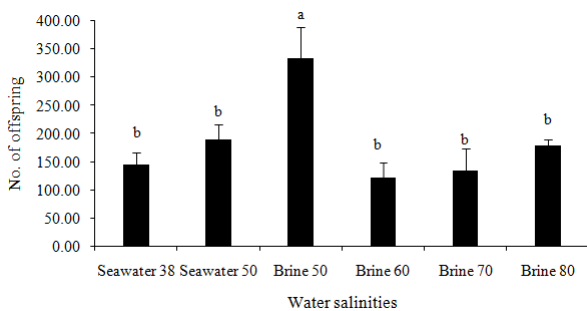


Figure 5. The relation between the different water salinities and the offspring per female, where rice bran was used as a food. Data expressed as mean ± SE. Error bars represent the standard error of the mean. Bar columns having different letters are significantly different at P<0.05 (Tukey test)

**Inter-brood interval**

The inter-brood interval taken by the *Artemia* individuals, fed on rice bran and reared at different water salinities did not exhibit a considerable variation (Tukey, P>0.05), see Figure 6. It ranged from 2.20 to 4 days for all treatments.

**The percentage offspring encysted**

According to the percentage of offspring encysted, the findings exhibit a variation within the different treatments. The brine water of 70 ppt had the peak proportion of offspring that have turned to cysts, with a percentage of 72.42%, followed by brine water of 60 ppt. On the contrary, the lowest rate of encysted offspring was in brine water 80 ppt, 17.48%, as seen in Figure 7.

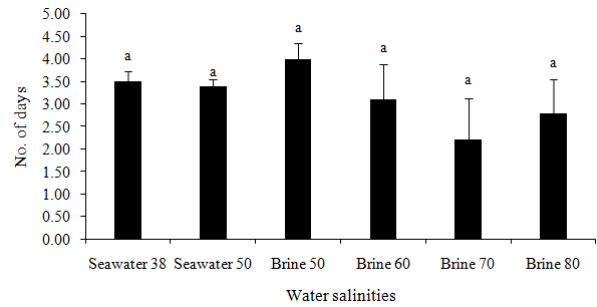


Figure 6. The effect of water salinities on the inter-brood interval where rice bran was fed. Data represented as mean ± SE. Error bars represent the standard error of the mean. Bar columns having different letters are significantly different at P<0.05 (Tukey test)

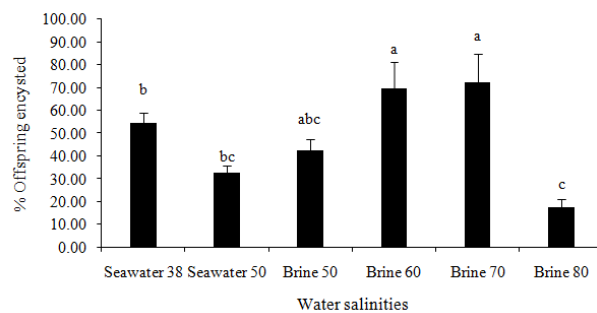


Figure 7. The % offspring encysted produced by *Artemia franciscana* individuals cultivated in the different water salinities fed on the rice bran particles. Data expressed as mean ± SE. Error bars represent the standard error of the mean. Bar columns having different letters are significantly different at P<0.05 (Tukey test)

**The lifespan for reproductive females**

For the lifespan of reproductive females, the results reveal no considerable variation between the *Artemia* individuals grown under the various water salinities fed on rice bran. The reproductive females lived for a number of days ranging from 32 to 37 days, as illustrated in Figure 8. It does not appear that the lifespan of the *Artemia* female is a linear function of the salinity.

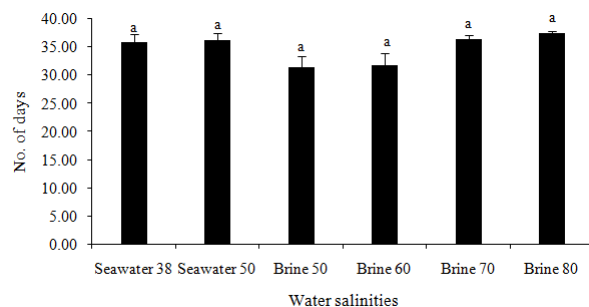


Figure 8. The lifespan of the reproductive females of *Artemia franciscana* grown in different water salinities and fed on rice bran. Data represented as mean ± SE. Error bars represent the standard error of the mean. Bar columns having different letters are significantly different at P<0.05 (Tukey test)

**Male lifespan**

The lifespan of the males of *Artemia* individuals did not exhibit a significant difference under the various wa-

ter treatments; results are shown in Figure 9. The average male lifespan ranged between 29.40 and 38 days.

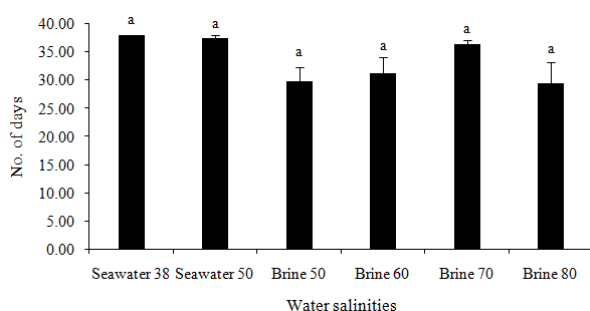


Figure 9. The impact of water salinities on the male lifespan of *Artemia franciscana* that were growing by feeding on rice bran. Data expressed as mean  $\pm$  SE. Error bars represent the standard error of the mean. Bar columns having different letters are significantly different at  $P < 0.05$  (Tukey test)

## Discussion

The pre-reproductive period was long with higher salinities of 60, 70, and 80 ppt, comparable with Abatzopoulos et al. (2003), who claimed that the length of the pre-reproductive phase lengthened as salinity increased. Also, Agh et al. (2008) mentioned that the salinity significantly impacted the pre-reproductive stage, with an associated increase in salinity. There are no significant differences among the seawater 38, seawater 50, and brine water 50 ppt, and within the brine water of 60, 70, and 80 ppt.

We found that the reproductive period was longer in higher salinities than in lower salinities. Similar to Browne and Wanigasekera (2000), who indicated the proportional increase of the female reproductive period with the increasing salinity. There are no significant differences between the results of lower salinities.

However, there is no significant difference between the offspring per brood. Some research mentioned an optimum combination of temperature and salinity for the offspring per brood that differs among various *Artemia* species among the different water salinities (Agh et al., 2008). This reaction between multiple factors has to be investigated further (Abatzopoulos et al., 2003). However, some research revealed that the salinity does not impact the number of broods (Abatzopoulos et al., 2003; Agh et al., 2008); there is a slight difference in the number of broods in the various salinities.

Similar to the offspring per brood, there is no significant difference between the various treatments in the number of offspring per female, except for the brine water 50 ppt treatment. These results emphasize that the reproduction output does not relate to the salinity, although the reproduction might be optimum at a specific temperature–salinity combination (Abatzopoulos et al., 2003; Agh et al., 2008).

There is no significant difference in the inter-brood interval in the various salinities. Consequently, we could conclude that the salinity does not impact the periods between broods. This finding has also been claimed by Agh et al. (2008) that salinity has little effect on the time between broods.

The graph shows that the percentage of encysted offspring increased from low to higher salinities (50, 60, and 70 ppt). These results agree with the theory that the increasing salinity represents an environmental factor that leads to the oviparity reproduction method (Abatzopoulos et al., 2003; El-Bermawi et al., 2004). Results illustrate that the lifespan of reproductive females and the male lifespan were not affected by the various water salinities.

Rice bran is a by-product that is widely available and cheap, and is used as an appropriate feed for *Artemia* cultivation in intensive systems (Benedictal et al., 2009). It has high contents of protein, amino acids, lipid, and vitamin B (thiamine) (Ullimaz et al., 2020). It improves the biological characteristics of *Artemia* (Balachandar and Rajaram, 2019). It has been used as feed for *Artemia* in many researches (Balachandar and Rajaram, 2019; Lavens and Sorgeloos, 2018; Le et al., 2019; Sivaji, 2016; Sorgeloos, 1980). We observed that the highest offspring encysted due to the oviparity approach in 70 and 60 ppt brine water. However, it should be considered that rice bran induces the oviparity mode (Vahdat and Oroujlou, 2021). Further research is required to identify the optimum conditions (temperature, water salinity, oxygen level) to determine the better conditions to simulate the oviparity mode in *Artemia* growing in desalination brine water.

*Artemia* has an effectual osmoregulatory approach to preserve the water quantity of the homeostasis inside its body (Sserwadda et al., 2018; Van Stappen, 2003). It exerts some energy to adapt to the higher salinities; other metabolic and physiological processes, including growth and reproduction, may be affected (Dana and Lenz, 1986; Sserwadda et al., 2018; Van Stappen, 2003). We have observed longer pre-reproductive and reproductive periods in higher salinities. Besides, offspring per brood, broods per female, and offspring per female were low in the rising salinities. These indicators are comparable with Sserwadda et al. (2018). They found that the descending fecundity and more extended pre-reproductive period resulted from higher energy costs for osmotic homeostasis in increasing salinities. In addition, the energy cost of osmoregulation differs according to the salt composition (Sserwadda et al., 2018; Van Stappen, 2003).

## Conclusion

The data derived from the study of reproductive characteristics of *Artemia* provide evidence that the brine water disposed of from desalination plants can be used to grow *Artemia* till the adult stage and be an appropriate environment for the *Artemia* biomass and cyst production. In addition, the rice bran is an available source that

could be used to feed *Artemia* to the adult stage in the desalination brine water. Using other diets and other diet combinations are recommended to assess the optimum feed under the desalination brine conditions.

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