

Effect of different Salinity levels on the Hatchability and Survival of Brine Shrimp, *Artemia salina* (Linnaeus, 1758) from Malindi, Kenya

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ABSTRACT

Brine shrimp, *Artemia salina* (Linnaeus, 1758) has become popular in aquaculture industry as live feed to over 85% of the cultured species around the world. *A. salina* is a non-selective filter-feeder of high nutritive value which thrives in a wide range of water quality variables under high density, thus it is suitable feed in fin-fish culture. The stocking density in each hatching systems were 700 cysts litre⁻¹, replicated three times. The concentration in hatching tanks was changed every two days. The treatments were maintained at (4 ppt saline solution (freshwater), 28 ppt saline solution (brackish water) and 40ppt saline solution (seawater)) and the pH between 7.8-8.5, 8.2-8.9 and 8.5-9.3 among treatments respectively. A uniform temperature of 28°C was maintained by thermostat for all the treatments throughout the study period. Non-parametric tests, Friedman's analysis method were used for hatchability and incubation period and Probit Analysis method used for survival data analysis. Hatchability of cysts was not significantly different among treatments ($p > 0.05$). However, there was significant difference in the hatching period as compared to salinity levels with the ($p = 0.029$). Moreover, there was significant difference in the salinity levels on survival of *A. salina* nauplii cultured for five days with the goodness-of-fit tests ($p < 0.05$) and the test for equal slopes were significant, the comparison of survival were not similar regardless of the salinity Levels.

Keywords: *Artemia salina*, hatchability, survival, Friedman's analysis, Probit analysis.

INTRODUCTION

The brine shrimp, *Artemia salina* (Linnaeus, 1758) is important as a live feed for larval fish in over 85% of cultured species around the world (Soundarapandian and Saravanakumar, 2009). It is easy to handle, adaptable to wide environmental conditions, non-selective filter feeder and capable of growing at very high densities. Moreover, it also has high nutritive value, food conversion efficiency, fecundity, short generation time, and a long life span. *A. salina* is distributed mostly in hypersaline lakes, brine ponds and lagoons. Brine shrimp thrive very well in natural seawater and can tolerate the salinity ranges from 3 to 300 ppt, is used to test for toxins, and also as a live feed for fish hatchlings (Lavens and Sorgeloos, 1996) and this usefulness is based on its portability, adaptability to wide environmental conditions, non-selective filter feeding and ability to grow at very high densities. The use of *A. salina* as a food for the larviculture of aquatic species began in the 1930's (Sorgeloos *et al.*, 2001). However, providing adequate amount of *A. salina* cysts and biomass hampers the conservation of crustaceans, and freshwater and marine fish (Lavens and Sorgeloos, 1996). Current strategy for sustainable supply of *A. salina* is the storage of its cysts for artificial hatching in hatcheries when required to feed fish hatchlings. Good quality of *A. salina* cysts is maintained by reduction of their water content to lower than 4%, and avoiding direct exposure to sunlight and oxygen (Vanhaecke and Sorgeloos, 1982; Bosteels *et al.*, 1996). To avoid the predation problem the culture should be performed in controlled conditions. While sustainable supply of *A. salina* can be achieved by hatching of cysts, it is not clear what optimum salinity is required for proper hatching and survival.

Survival of hatchlings for fish species such as the African catfish, Nile tilapia or Goldfish is often low mainly due to inadequate availability of the right type of live feed (FAO, 2012). A live feed is required by hatchlings soon after the absorption of the yolk sac, to nourish the fish for energy and growth, since the digestive system is not yet well formed. Common ways of producing live feed on farms involve fertilizing ponds with organic manure, for the proliferation of zooplankton, which are and fed to the catfish fry. However, this is laborious, takes time before the live feed can grow to sufficient amounts, and the quality of the feed is not certain. Furthermore, it is susceptible to

excessive fertilization, which impacts on water quality in ponds. Therefore, the use of *A. salina* cysts as sources of shrimp that is given as live feed is a feasible alternative. Various methods of using *A. salina* as feed are employed in both fish (Sorgeloos *et al.*, 2001; Celada *et al.*, 2007) and crustacean (Sorgeloos *et al.*, 1998; Naegel and Rodriguez-Astudillo, 2004) culture. Apart from its high nutritive value, short generation time, and high fecundity, *A. salina* cysts also hatch within a short interval of about 24 hours, and so could be made available to the fry at short time. However, it is uncertain at what salinity levels the hatchability and survival of the shrimp would be optimum, to enhance benefits from the available cysts of *Artemia*. This study investigated hatching rate, hatchability and survival of the brine shrimp at three salinity levels, in order to determine optimum conditions under which the cysts should be incubated.

MATERIALS AND METHODS

The study was conducted at the Department of Fisheries and Aquatic Sciences hatchery, University of Eldoret, Kenya at altitude of 2140m above sea level. The cysts were sourced from Malindi salt works in an air-tight polyethylene bag and were refrigerated for one week in the laboratory before experiment.

A five day experiment was conducted in the hatchery and repeated four times during the study period. Thus, 2 glass compartmented aquaria each of 90 litres volume with illumination of 40 watts with three replicates were set in the hatchery and temperature set at 28°C using the sterilized thermostat. Aeration hose and air-stones were sterilized in sodium hypochlorite solution and thereafter rinsed with tap water and left to sun-dry for 2 hours before commencement of the experiment. 10 litre of saline water was put in each compartment. The dilution was at the rate of 4g, 28g and 40g of common salt (NaCl) each in a litre (1,000 ml) of water respectively. 7,000 cysts, with a stocking density of 700 cysts per litre of water were put in compartment of 0.4%, 2.8% and 4.0% saline solutions. Hatchlings were counted after 24 hours. Water was changed in the system after every two days.

Data was collected at 6, 12, 18 and 24 hours intervals. The hatchlings were calculated using formula; (Hatched cysts = stocked cysts – unhatched cysts). The hatchlings transferred into different treatments for specific treatments to determine the survival rate of hatchlings.

The nauplii were kept for 5 days in different salinity levels to determine survival rate. Nauplii were fed after 12 hours with microalgae, bacteria, and detritus. Survival rate was calculated by formula; (survival rate = stocked nauplii -dead nauplii).

RESULTS

Hatching Rate

Changes in hatching rate during experimental periods are shown in Table1. There was no significant difference in hatching rate during experimental period in treatment 1 as compared to control. In treatment 2 there was significant difference in hatching rate between 6 and 24 hours periods but not significant at 12 and 18 hours while in treatment 3 hatching rate was highly significant within 12 hours period and was significant at 18 and 24 hours. Among treatments there was significant difference in hatching rate at 6, 18 and 24 hours period in treatment 1but not at 12 hour period. At 18 hours there was significant difference between treatment 3 and treatment 1 and 2

Table 1: Hatching rate of *A. salina* cysts in different salinity levels for 24 hour experiment period

		Parameters				
Treatments	stocking density	0 hours	6 hours	12 hours	18 hours	24 hours
Treatment 1(4ppt)	7000	0±0 ^a	1995±82 ^a	2478±88 ^a	2792±126 ^a	3720±82 ^a
Treatment 2 (28ppt)	7000	0±0 ^a	922±71 ^b	2193±235 ^a	2900±186 ^a	5888±199 ^b
Treatment 3 (40ppt)	7000	0±0 ^a	618±171 ^c	2252±177 ^a	4262±236 ^b	6292±157 ^c

Figures with differing superscript within each parameter in columns were significantly different (P = 0.001).

Hatchability

The differences in the mean number of nauplii hatched in three salinities were insignificant at 4.0% saline solution (6292 nauplii), while it was lowest at 0.4% saline solution (3720 nauplii). The variations in the means were highest at 2.8% saline solution (±199 nauplii) with the lowest being observed at 0.4% saline solution (±82). The mean

hatchability for the two salinity levels; 2.8% and 4.0% saline solutions were not significantly different at 5988 and 6292; with S.E.M at 109 and 157 respectively as shown in table 2.

Table 2: Hatchability of *A. salina* in different salinity levels

Parameter	0.4% saline solution	2.8% saline solution	4.0% saline solution	P-Value
Hatchability	3720±82 ^a	5888±199 ^b	6292±157 ^c	0.029

The test statistic, $S=9.00$, has a p-value of 0.029. For confidence level of 0.05, there is significant difference in the hatching rate on different salinity levels (table 2).

Survival

The initial surviving nauplii in 0.4% saline solution started low at 3270 nauplii, 2.8% saline solution at 5988 nauplii and 4.0% saline solution highest at 6292 nauplii. The survival curve was a negative curve as mortalities reduced the number of individuals in the treatments. The highest survival was achieved in 4.0% saline solution with 5418±203 of nauplii surviving after five days experiment period. 2.8% saline solution was next at 4631±303, while least survivals were observed at 0.4% saline solution with 2441±266 (Table 3).

Table 3: Survival of *A. salina* nauplii in different treatments for five days experiment period

Parameter	0.4% saline solution	2.8% saline solution	4.0% saline solution	P-Value
Survival	2441±266a	4631±303b	5418±203c	0.0001

The goodness-of-fit tests ($P = 0.000, 0.000$) and the probability plot suggest that the Weibull distribution does not fit the data adequately. Since the test for equal slopes was significant ($P = 0.000$), the comparison of mortalities of *A. salina* was not similar regardless of the salinity levels. The salinity levels of 0.4%, 2.8% and 4.0% were significantly different because the coefficient associated with 4.0% saline solution and 0.4% saline solution was significantly different from 2.8% saline solution ($P = 0.000, 0.000$). At 35ppt, 69% of survivals in 4.0% saline solution, 53% of survivals in 2.8% saline solution and 20% of survivals in 0.4% saline solution occur beyond day 6. 0.4% saline solution had the highest potency for mortality followed by 2.8% saline solution and least potency in 4.0% saline solution. The maximal efficacy was equal for all the treatments. The table of percentiles shows that 50% of *A. salina* mortalities occurred after 22 hours, 26 minutes in 0.4% saline solution; after 9 days in 2.8% saline solution and after 50 days in 4.0% saline solution (Fig 1).

Test for equal slopes: Chi-Square = 284.461 $n = 3$ P-Value = 0.0001

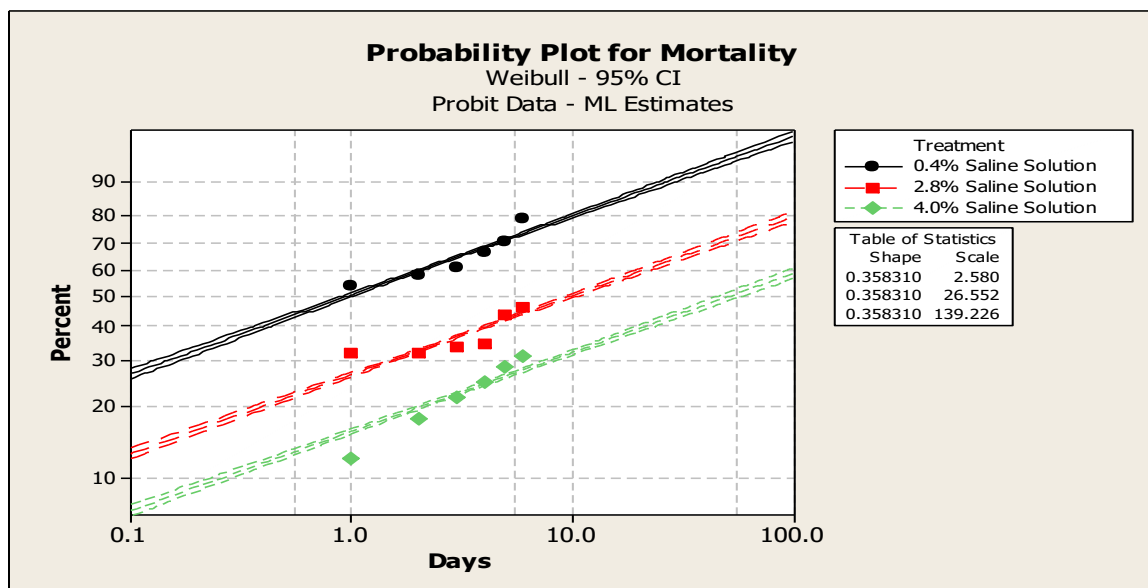


Figure 1: Probability analysis plot for survival of *A. salina* nauplii in different treatments

DISCUSSIONS

Quantitative effects of salinity on cyst hatching are related in the first place with the hydration-level that can be reached in the cysts. Above a threshold salinity, insufficient quantities of water can be taken up to support the embryo's metabolism. This threshold varies among strains of *Artemia* (Kulasekarapandian and Ravichandran, 2003). Secondly the incubation salinity will interfere with the amount of glycerol that needs to be built up to reach the intra-cystic osmotic pressure (Versichele and Sorgeloos 1980). The fastest hatching rates will thus be achieved at the lowest salinity levels since it will take less time to reach breaking. When considering high salinity levels, it is very likely that cysts from different geographical origin contain insufficient quantities of carbohydrates to meet their varying hyperosmotic requirements (Versichele, 1983). As a result optimal artificial seawater salinity for cyst hatching varies among strains and environmental conditions in *Artemia*; in a range of 5-70‰ (Versichele and Sorgeloos, 1980). Comparative studies of the hatching behaviour of cysts of different origin show a considerable variation in hatching percentage, rate and efficiency (Lavens, 1981). However, none of these parameters is strain specific as they are influenced by a wide array of factors like harvesting, processing, storage and hatching techniques, as well as production conditions affecting the parental generation (Emslie, 2012). For optimal use of *Artemia* in aquaculture the hatching characteristics of any batch of cysts being used should be known (FAO, 2012). There was general increase in the hatchability of *Artemia* cysts in different salinity levels. Treatment 2 had an exponential hatchability curve while treatment 3 had a linear curve. At the first six hours, treatment 1 had a higher hatchability followed by treatments 2 and 3 had less hatchability. At 24hrs the treatment 3 had higher hatchability with treatment 2 coming close second while treatment 1 had overall lower hatchability. This could have been because of diffusion gradient of 4ppt is higher while least in higher salinity levels thus the hatching starts at a higher rate in least salinity concentrations. But with time the hatchability in the least salinity reduces as the higher salinity levels increases in the hatching of the cysts. This could be attributed to the effect of salinity on triggering metabolism of the embryo and dissolving the shells; up to a certain level of salinity. The test statistic, S, has a p-value of 1.000. For confidence level of $\alpha=0.05$, there is insufficient evidence to reject null hypothesis because the p-value is greater than the confidence level. Therefore there was no significant difference in the salinity levels and hatchability.

In this study, temperature was uniform (28°C) for all the three salinity levels tested and pH of the rearing medium was 8.15 ± 0.35 , 8.55 ± 0.35 and 8.9 ± 0.4 for 0.4%, 2.8% and 4.0% saline solutions respectively. Water temperature and pH is probably the most important environmental variables in *A. salina* cultures, because it directly affects metabolism, oxygen consumption, growth, moulting and survival (Herbst, 2001). Consequently, 0.4%, 2.8% and 4.0% saline solutions were significantly different because the coefficient associated with 4.0% and 0.4% saline solutions was significantly different from 2.8% saline solution ($P=0.000, 0.000$). The table of percentiles shows that 50% of *A. salina* mortalities occurred after 22 hours, 26 minutes in 0.4% saline solution; after 9 days in 2.8% saline solution and after 50 days in 4.0% saline solution. So the nearest point to 35ppt which is the salinity level of marine environment is at 28ppt which was found to be ideal for culturing *A. salina* nauplii. At 35ppt, 69% of survivals in 4.0% saline solution, 53% of survivals in 2.8% saline solution and 20% of survivals in 0.4% saline solution were observed beyond day 6. 0.4% saline solution had the highest potency for mortality followed by 2.8% saline solution and least potency in 4.0% saline solution. The maximal efficacy was equal for all the treatments. This suggests that higher salinity is important for better survival even though *A. salina* is euryhaline (FAO, 2012). Best results for survival and growth of the San Francisco strain of *A. salina* were found to be about 60ppt (Douillet, 1987), compared to 4ppt salinity levels which gave lower survival. Under laboratory conditions 13 geological strains of *A. salina* (Vanhaeke *et al.*, 1984) had high survival over a wide range of salinity levels (35-100ppt). Triantaphyllidis *et al.*, (1995) reported that a parthenogenetic population of *A. salina* did well at salinity levels of 60 ppt and 100 ppt while at 35, 40 and 80 ppt the survival was less than 50% after 27 days of culture.

CONCLUSIONS

From this study it could be concluded that seawater salinity, 4.0% saline solution was highly suitable for the culture of *A. salina* as evidenced by higher hatchability and survival (>60%). There was no significant difference in the hatchability of *A. salina* cysts reared under different salinity conditions. Survival improved with higher salinity levels, and we may conclude that higher salinity could enhance survival of *Artemia*.

A. salina cysts should be hatched in a sterilized saline water of between 25-33ppt to get the highest hatchability. Moreover, the hatching period should be 24 hours to get the most of the cysts hatched. Finally, the best survival rate is most probably achieved at 28ppt. Therefore; aquaculture farmers should be advised on using 4.0% saline

solution for hatching and culture of live feeds to improve on the larviculture of finfish and crustaceans in their farms.

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