EFFECT OF DIFFERENT SALINITIES ON THE HATCHABILITY AND SURVIVAL OF BRINE SHRIMP, *ARTEMIA SALINA* (LINNAEUS) FROM MALINDI, KENYA

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Abstract

The brine shrimp, *Artemia* has become popular in aquaculture industry as live feed to over 85% of cultured species around the world. Hatchability and survival of *Artemia* nauplii were tested in three different salinities. The stocking density in each hatching systems were 700 cysts/litre, replicated three times. Prior to hatching *Artemia* cysts, algae were cultured in separate culture jars for feeding right from nauplii stage. Water in hatching tanks was changed every two days. The salinities of culture systems were maintained at 4 ppt tap water (freshwater), 28 ppt brackish water and 40ppt seawater. The pH of the freshwater, brackish water, and seawater ranged between 7.8-8.5, 8.2-8.9 and 8.5-9.3 respectively. A uniform temperature of 29°C was maintained for all the culture systems throughout the study period. Minitab Release 14.0 Statistical Software was used to analyze data at 0.05 confidence level. Non-parametric tests, Friedman’s analysis method were used for hatchability and incubation period and Probit Analysis method used for survival data. 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 *Artemia* salina nauplii cultured for five days with the goodness-of-fit tests (P<0.05) and the test for equal slopes significant at P=0.000, the comparison of survival were not similar regardless of the salinity Levels.
Introduction

The brine shrimp, *Artemia* is a crustacean important as a live feed for larval fish in over 85% of cultured species around the world. It is easy to handle, adaptable to wide environmental condition, non-selective filter feeder and capable of growing at very high densities. Moreover, it also has high nutritive value, high food conversion efficiency, short generation time, high fecundity and a long life span. The use of *Artemia* as a food for the larviculture of aquatic species began in the 1930's. However, providing adequate amount of *Artemia* cysts and biomass hampers the conservation of crustaceans, and freshwater and marine fish (Lavens and Sorgeloos, 1996). Current strategy for sustainable supply of *Artemia* is the storage of *Artemia* cysts for artificial hatching in hatcheries when required to feed fish hatchlings. Good quality of *Artemia* 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).

*Artemia* 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. To avoid the predation problem the culture should be performed in controlled conditions. While sustainable supply of *Artemia* can be achieved by hatching of cysts, it is not clear what optimum salinity is required for proper hatching and survival. *Artemia salina* (L), widely distributed in salt pools and salt lakes of high salinities, is used to test for toxins, and also as a live feed for fish hatchlings. This usefulness is based on its portability, adaptability to wide environmental conditions, non-selective filter feeding and ability to grow at very high densities.

Survival of hatchlings for fish species such as the African catfish, Nile tilapia or goldfish is often low. A major factor underlying this low survival is inadequate availability of the right type of live feed. 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 harvested 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 *Artemia* cysts as sources of shrimp that is given as live feed is a feasible alternative. Various methods of using *Artemia* 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, *Artemia* cysts also hatch within a short interval of about 24 hours, and so could be made available to the fry at short notice. However, it is uncertain at what salinities the hatchability and survival of the shrimp would be optimum, to derive maximum benefits from the available cysts of *Artemia*. This study investigated hatchability and survival of the brine shrimp at three salinities, in order to determine optimum conditions under which the cysts should be incubated.

**Materials and Methods**

**Study area**

The study was conducted at the University of Eldoret’s Fisheries and Aquatic Sciences department hatchery in the months of January and February. University of Eldoret fish hatchery is located within the main campus at 00, 320N 35, 120E at altitude of 2140m above sea level. The area receives a mean annual rainfall of 1124mm with temperature ranging between 16.50c and 21.80c.

**Source of cysts**

The cysts were sourced from the Kenya Marine and Fisheries Research Institute, Mombasa in an air-tight polyethene bag. Cysts were refrigerated for one week at the Fisheries and Aquatic Sciences department. Aeration lines and stones, thermostat, glass aquaria and the lighting system for the project at the hatchery were sterilized in sodium hypochlorite solution (jik) rinsed with freshwater and left to sun-dry for 2 hours before commencement of the experiment.

**Experimental set-up**

The experimental set-up was made in triplicate in two partitioned aquaria. They were filled in each partition with 10L of tap water which had been kept overnight in tanks in the hatchery for
chlorine to diffuse out. The cysts were counted at 700l⁻¹ of water, bringing the total population to 7000 in every 10 litre compartment.

**Experimental design**

Cysts were hatched in 0.4%, 2.8% and 4.0% saline solutions, prepared by dissolving corresponding grams of salt, in a litre of water. The dilution was at the rate of 4g, 28g and 40g of common salt (NaCl) each in a liter (1,000 ml) of water respectively. Cysts were then bubbled in the solutions with an illumination of 40 watts bulb throughout the entire period of the experiment. Thermostats in each treatment were set at 28°C. the two aquaria with a total of 12 compartments; six compartments per tank were used. Each compartment had a total volume of 15 litres of water. 10 litre water capacities was put in each compartment and then their corresponding salinities dissolved in them by aerating them and raising the temperature to 28°C until total dissolution was achieved before cysts were hatched in them. Each compartment had 7,000 cysts, with a stocking density of 700 cysts per litre of water.

Each of the three treatments had three replicates distributed randomly in every compartment (i.e. three incubation tanks for each treatment). Cysts hatched in 24 hours. Each tank had water changed in the system after every two days up to the fifth day. The project was repeated four times in order to get the average of the effects variables of the research.

**Data collection**

Data was collected at a 6 hour interval up to the 24th hour when no more hatching occurred. The numbers of unhatched cysts were counted and then deducted from the original stocking density to get the total number of hatched cysts per treatment. After hatching treatments, the number of hatchlings were counted and transferred into fresh salty water treatments for specific treatments to study the survival of hatchlings.

The nauplii were kept for 5 days in their specific treatments to determine their survival rate. There was no extraneous feeding for the first 12 hours before complete absorption of yolk sac. Counting of nauplii was performed each day to ascertain the number of nauplii that survived for that day. After that they were fed on microalgae, bacteria, and detritus sourced from the fish farm.
ponds. At the 5th day the number of surviving nauplii was counted against the total mortalities, to determine survival of nauplii.

**Data analysis**

Descriptive and exploratory summary and inferential statistics were generated to analyse the data. Line graphs and bar-graphs of mean values with standard errors were computed, estimate medians were also calculated and tables generated for the statistics. Non-parametric Friedman test (an alternative of two-way analysis of variance) was used to test for any significant difference in the hatchability versus salinity level at different time intervals. Probit analysis was further used to test the hypothesis of no difference in survival rate of *Artemia salina* (*L*) nauplii on different salinity conditions.

The entire graphical summary and tabled done with Microsoft Excel spreadsheet while descriptive and inferential statistics were done by Minitab 14 statistical software.

Friedman`s analysis formulae is: 

\[ S = 12[nk(k+1)]^{-1} \left[ \sum \left( \sum R_j \right)^2 \right] - 3n(k+1) \]

Where; \( j = 1, 2, 3, \ldots, k \)

\( K = \) the number of treatments conditions

\( N = \) the number of blocks

\( R_j = \) the sum of ranks for treatment \( j \).

While Probit analysis of the formulae \( \pi_j = c + (1 - c) g(\beta_0 + x_j \beta) \) was used to analyze survival at confidence level of \( P=0.05 \).

**Results**

**Hatching period**

The test statistic, \( S = 9.00 \), has a p-value of 0.029. For confidence level of 0.05, there is sufficient evidence to reject \( H_0 \) because the p-value is less than the confidence level. Therefore there is significant difference in the hatching period on different salinity levels (table 1).
<table>
<thead>
<tr>
<th>Time</th>
<th>N</th>
<th>Estimated Median</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>3</td>
<td>941</td>
<td>3.0</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>2192</td>
<td>6.0</td>
</tr>
<tr>
<td>18</td>
<td>3</td>
<td>3106</td>
<td>9.0</td>
</tr>
<tr>
<td>24</td>
<td>3</td>
<td>5715</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Grand median = 2989, S = 9.00, DF = 3, P = 0.029

From table 1, the significant difference can be seen from the estimated median values which are which have a big variance. This can also be shown by general increase in the hatchability of *Artemia* cysts in different salinities (figure 1). The 28 ppt had an exponential hatchability curve while 40ppt had a linear curve. In the first six hours the 4ppt salinity had a higher hatchability at 1710 nauplii followed by 28 ppt at 790nauplii, while 40ppt salinity had less hatchability at 530 nauplii. At 24hrs the 40ppt had higher hatchability at 6292nauplii with 28ppt coming second at 5988 nauplii while 4ppt had lower hatchability at 3720 nauplii.
The hatchability trend of *Artemia salina* under different salinities.

**Hatchability**

The test statistic, S, has a p-value of 1.000. For confidence level of 0.05, there was insufficient evidence to reject $H_0$ because the p-value is greater than the confidence level. Therefore there is no significant difference in the salinity levels and hatchability (table 2).

**Table 2: Friedman Test - Hatchability versus Salinity level blocked by Time**

<table>
<thead>
<tr>
<th>Salinity level</th>
<th>N</th>
<th>Estimated Median</th>
<th>Sum of Ranks</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4</td>
<td>2509.3</td>
<td>8.0</td>
</tr>
<tr>
<td>28</td>
<td>4</td>
<td>2513.0</td>
<td>8.0</td>
</tr>
<tr>
<td>40</td>
<td>4</td>
<td>2832.7</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Grand median = 2618.3, $S = 0.00$, $DF = 2$, $P = 1.000$
Further descriptive analysis shows the insignificance in the mean number of nauplii hatched was highest at 40ppt (6292 nauplii), while it was lowest at 4ppt (3720 nauplii). The variations in the means were highest at 28ppt (±487 nauplii) with the lowest being observed at 4ppt (±200). The mean hatchability for the two salinities; 28 and 40ppt were 5988 and 6292; with standard deviation at 487 and 384 respectively as shown in figure 2.

![Figure 2: the mean number of Artemia salina nauplii hatched at different salinity levels.](image)

**Survival**

The goodness-of-fit tests (p-values = 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 is significant (p-value = 0.000), the comparison of survival will not be similar regardless of the salinity levels (there was significant difference in survival as compared to the different salinity levels).

**Probit Analysis: Survival, trials versus Treatment, Days**
Test for equal slopes:  Chi-Square = 811.868, DF = 5, P-Value = 0.000

Figure 3: Probability analysis plot for survival of Artemia salina (L) nauplii under different salinity levels.

The initial surviving nauplii for 4ppt started low at 3270 nauplii, 28ppt at 5988nauplii and 40ppt highest at 6292nauplii. The survival curve was a negative curve as mortalities reduced the number of individuals in the treatments. Relatively the survival rate at 40ppt was highest at 4808±138 nauplii, followed by 28ppt at 3738±215 nauplii and least survivals at 4ppt (1435±72 nauplii). The figure 4 shows the trend of survival for the five day experimental period.
Figure 4: The survival rate of *Artemia salina* nauplii kept in different salinity levels for five days.

The highest survival was achieved in 28ppt with 88.51% of nauplii surviving after five days experiment period. 40ppt was next at 78.05%, while least survivals were observed at 4ppt with 44.55%. The variations in these survivals were however different with 28ppt having ±11.18% variations, closely followed by 4ppt at ±8.91% and lastly 40ppt had the least variations at ±4.17% (Figure 6).
Figure 5: The percentage survival of Artemia salina nauplii on different salinity levels for five days treatment period.

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. In this case, the salinity levels (4ppt, 28ppt and 40ppt) are significantly different because the coefficient associated with 28ppt (control) is significantly different compared with 4ppt and 40ppt at days 1, 2, 3, 4 and 5 (p-value = .000). The table of percentiles shows that 50% of survivals were observed at 22.9643 salinity levels. So the nearest point is at 28ppt which is ideal for culturing Artemia. 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. The fastest hatching rates will thus be noted at the lowest salinity levels since it will take less time to reach breaking. When considering high salinities, 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, 1983). 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 salinities. The 28 ppt had an exponential hatchability curve while 40ppt had a linear curve. At the first six hours, 4ppt had a higher hatchability followed by 28 ppt and 40ppt had less hatchability. At 24hrs the 40ppt had higher hatchability with 28ppt coming close second while 4ppt had overall lower hatchability. This could have been because of diffusion gradient of 4ppt is higher while least in higher salinities 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 salinities 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 $H_0$ because the p-value is greater than the confidence level. Therefore there was no significant difference in the salinity levels and hatchability.

In the present study, temperature was uniform (28°C) for all the three salinities tested and pH of the rearing medium was 8.15±0.35, 8.55±0.35 and 8.9±0.4 for 4‰, 28‰ and 40‰ respectively. Water temperature and pH is probably the most important environmental variables in *Artemia* cultures, because it directly affects metabolism, oxygen consumption, growth, moulting and survival (Herbst, 2001). Higher survival was obtained on the *Artemia* cultured in 40 ppt (80%) followed by 28-34ppt (75%) and 2-4ppt (30%). This suggests that higher salinity is important for better survival even though *Artemia* is euryhaline. Best results for survival and growth of the San Francisco strain of *Artemia* were found to be about 60ppt (Douillet, 1987), compared to 4ppt salinities which gave lower survival. Under laboratory conditions 13 geological strains of
*Artemia* (Vanhaeke *et al.*, 1984) had high survival over a wide range of salinities (35-100ppt). Triantaphyllidis *et al.* (1995) reported that a parthenogenetic population of *Artemia salina* did well at salinities 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 the present study it could be concluded that seawater salinity, 40ppt was highly suitable for the culture of *Artemia* as evidenced by higher hatchability and survival (>80%). There is no significant difference in the hatchability of *Artemia salina* (*L*) cysts reared under different salinity conditions. Survival improved with higher salinities, and I conclude that higher salinity could enhance survival of *Artemia*.

**Recommendations**

*Artemia 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. so aquaculture farmers should be advised on the best ways of hatching and survival of live feed hatching and subsequent culture to improve the larviculture of finfish and crustaceans in their farms.

**References**


