West African Journal of Fisheries and Aquatic Sciences, 2(1), 9-21 (2021)
http://journals.unilag.edu.ng/index.php/wajfas/article/view/1276
http://wajfas.unilag.edu.ng, http://journals.unilag.edu.ng/index.php/wajfas

Research Article

Survival and Reproductive Responses of Four *Artemia* Strains in Low Salinity

Richard L. Bwala[1*], Khalid Salie[2] and Gilbert Van Stappen[3]

[1] *National Institute for Freshwater Fisheries Research, New Bussa, Niger State, Nigeria,*
[2] *Department of Animal Sciences, Stellenbosch University, South Africa,*
[3] *Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Belgium*

*Corresponding author: richardl669@gmail.com

ABSTRACT

The brine shrimp, *Artemia* thrives in sea water salinity. In such condition, high survival and nauplii production rather than cysts should be expected. In this study, low salinity (0-32 gL^{-1}) was tested for its effect on survival and some reproductive traits of four *Artemia* strains: *Artemia franciscana* from the Great Salt Lake (GSL) and Vinh Chau as well as parthenogenetic strains from Tuz and Balikun. Survival at instar I and II developmental stages was tested in axenic condition over 48 h, while that of two inoculation ages (0 and 8 days) was tested in xenic condition over nine days. Reproductive traits were also assessed xenically. Results showed that in the xenic test, the GSL strain had significantly (p<0.05) higher survival at 20 and 32 gL^{-1}. No difference (p>0.05) was found between the two instar developmental stages tested. Similarly, no significant differences (p>0.05) were observed among the inoculation ages at the different salinities except at 5 gL^{-1}. The GSL strain had significantly (p<0.05) higher offspring produced as nauplii. We conclude that 20 gL^{-1} salinity and the GSL strain are the most suitable when considering biomass culture for the purpose of nauplii production as live food for aquaculture species.

Keywords: Artemia, Axenic, Inoculation age, Instar, Salinity, Strain, Xenic
Article History: Received 12 August 2021; Accepted 15 November 2021; Published 29 November 2021

INTRODUCTION

Artemia populations existing naturally in the wild inhabit environments with a wide range of salinity. *Artemia* have been found in salinities as low as 3 gL^{-1} and as high as 340 gL^{-1} (Gajardo & Beardmore, 2012). Consequently, their culture either in large artificial ponds for commercial purposes or in the laboratory for experimental purposes has also been conducted in media at varying levels of salt concentrations. Several investigations, particularly in terms of survival and growth characteristics have been conducted at ranges between sea water salinity to levels which are well above it (Browne & Wanigasekera, 2000; Abatzopoulos *et al*., 2003; El-Bermawi *et al*., 2004; Agh *et al*., 2008; Castro-Mejia *et al*., 2011; Aalamifar *et al*., 2014). Generally, *Artemia* perform efficiently at a salinity range 30 – 80 g L^{-1} (Lavens & Sorgeloos, 1991). Physiologically, *Artemia* has been regarded to thrive well at sea water salinity (Van Stappen, 1996a). Considering that high caloric cost for osmoregulation occurs at higher salinity (Wedemeyer, 1996), other physiological functions will consequently occur less optimally and conversely, better physiological performances are expected at low salinity.

Studies have shown that certain strains of *Artemia*, however, thrive better at low salinity. During a nine-day study for the combined effects of temperature and salinity on the survival of *Artemia* from various geographical origin, high survival was recorded at salinities ranging from 35 to 110 gL^{-1} in twelve out of thirteen strains tested. Significant differences in response to lower salinities between 5 and 15gL^{-1} revealed that some strains such as the *Artemia persimilis* strain from Buenos Aires, Argentina, could perform considerably better than the others tested (Vanhaecke *et al*., 1984). A laboratory study using parthenogenetic and bisexual *Artemia* from India (exact strains not specified) cultured over 20 days in freshwater (2 g L^{-1}), brackish water (28 – 33 gL^{-1}) and seawater (34 – 55 gL^{-1}) showed that high survival of both strains was achievable with the brackish water (75 % survival) and seawater (80 % survival), but survival was low in the freshwater (Soundarapandian & Saravanakumar, 2009). A parthenogenetic population in small temporary lagoons around Lake

Urmia, Iran, was monitored in the field over a period of two years, and was observed to reach maturity at very low salinity of 10 gL^{-1}, and to reproduce at salinity between 15 -20 gL^{-1} (Agh *et al*., 2007).

Within the overall scope of this research work, the culture of *Artemia* biomass for the purpose of nauplii production by utilizing the ovoviviparous reproductive mode was considered. *Artemia* nauplii are mainly used as live food for the larvae of many aquaculture species. Nauplii production using ovoviviparously reproducing biomass has previously been described, using a technique which involves diet manipulation, optimal culture water exchange and high constant oxygen levels (Lavens & Sorgeloos, 1987). The culture was conducted at 50 gL^{-1} salinity and the system yielded up to 30 g wet weight nauplii day^{-1} 100 L^{-1} culture tank at a stocking density of 5,000 adults L^{-1} over a culture period of 35 days. Therefore, the present study further considered culturing *Artemia* at low salinity. Contrary to the practice of culturing in high salinity for the purpose of cyst production (oviparity), low salinity was considered to support good physiological condition, and consequently to result in high survival and ovoviviparity.

In addition to the low salinity treatment and in order to ensure that high survival is achieved during culture, the test also considered the responses of *Artemia* at some selected growth stages. It has been reported that different growth stages in *Artemia* are affected differently by salinity (Clegg & Trotman, 2002). According to these authors, the enzyme Sodium Potassium Adenosine triphosphatase (Na, K-ATPase) is known to be involved with the osmoregulatory mechanism in both nauplii and adult stages of *Artemia*. However, the level of the enzyme found in the nauplii is based on a programmed mechanism which is independent of environmental salinity. This programmed mechanism has been considered to occur only for a short-term, mainly from the period of emergence of the embryo to the first nauplii development stage. In contrast to the apparently independent osmoregulatory mechanism of the nauplii, the Na, K-ATPase in adult *Artemia* seems to be influenced by the environmental salinity (Clegg & Trotman, 2002). Conversely, Vanhaecke *et al*. (1984) asserted that mortality rate in *Artemia* reduces as they grow to adult stages, while other studies (Wear & Haslett, 1986; Wear *et al*., 1986) reported that adult *Artemia* are more tolerant than juveniles. On these premises, two hypotheses were formulated. Firstly, we assumed that if the independent osmoregulatory mechanism in the nauplii is limited only to the instar I stage (Clegg & Trotman, 2002), then there is a likelihood of increased mortality rate when the instar II stage is used for inoculation of culture. Secondly, if mortality rate reduces in adult *Artemia* and if the adults are more tolerant than the juveniles (Vanhaecke *et al*., 1984; Wear *et al*., 1986), then it will be more beneficial to inoculate the culture with the adults rather than with the nauplii.

As salinity preference and tolerance in *Artemia* may be strain-specific and influenced by growth stages respectively, laboratory tests are indispensable before mass culture, with the aim to select a suitable low salinity level, an *Artemia* strain with high rates of survival and ovoviviparity, and an appropriate inoculation age of *Artemia*. The objective of the study was to investigate the effect of low salinity on the survival of a number of *Artemia* strains at different growth stages, and on their reproduction and life traits.

MATERIALS AND METHODS

A total of four strain, two strains belonging to the species *Artemia franciscana* (Kellogg, 1906) and two parthenogenetic strains were used in the study (Table 1). Cysts of the four strains were obtained from the cyst bank of the Laboratory of Aquaculture & *Artemia* Reference Center (ARC), Ghent University, Belgium. The cysts had been under storage in air tight packs under cold conditions (+4 ˚C).

Experimental Design

Three different experiments were conducted to test for the performance of *Artemia* at low salinities (0 – 32 gL^{-1}). In Experiment 1 and 2, *Artemia* were hatched in standard seawater salinity (32 gL^{-1}), and incubated into lower salinity at different developmental stages: in experiment 1 were instar I and instar II; in Experiment 2 were instar I and 8 days-old pre-adults. In both cases survival was recorded. In Experiment 3, animals from Experiment 2 (those exposed to low salinity from instar I onwards), after termination of the previous test, were taken for subsequent observation of their reproductive behaviour at low salinity.

Experiment 1: The effect of low salinity on the survival of instar I and II developmental stages of the different strains in axenic conditions

The survival of nauplii of the different *Artemia* strains at instar I and II developmental stages was tested at 0, 5, 10 and 32 g L^{-1} salinity over a 48 h experimental period, whereby the highest salinity was used as control. The different culture media (except 0 g L^{-1} which was fresh municipal water) were prepared by

Table 1: Species/strain, origin, reference number, mode of reproduction and abbreviations of *Artemia* used in the study. [1] = Van Stappen (2002)

Species / Strain	Country of origin	ARC reference number	Reproductive mode	Abbreviations Used
A. franciscana / Great Salt Lake	USA	1768	B[1]	GSL
A. franciscana / Vinh Chau	Vietnam	1787	B	VC
Parthenogenetic / Tuz	Kazakhstan	1761	P[1]	TUZ
Parthenogenetic / Balikun	China	1781	P[1]	BLK

B= Bisexual; P= Parthenogenetic

mixing natural sea water of 32 g L^{-1} salinity with fresh municipal water and the desired salinity was checked with a digital refractometer (Hanna Instruments: HI 96822) to the nearest 1 g L^{-1}.

The test was conducted axenically (Baruah *et al*., 2010). In order to maintain axenicity of cysts and nauplii, all manipulations were performed under a laminar flow hood. Sterilized Falcon tubes (FT) were used for hydrating the cysts and aeration was supplied through disinfected (using a solution of ethanol and denaturol) air tubes fitted with sterilized air filters and connected to an external air source. To neutralize the disinfectant, air was allowed to pass through the tubes for a few minutes. Air tubes were then inserted to the bottom of the FTs containing 18 mL distilled water, to ensure cysts were suspended within the water column during hydration and decapsulation. For each strain a small quantity of cysts (0.2 g) was added into the FTs and was allowed 1 h hydration period to ensure adequate decapsulation. In order to prevent pH from dropping below the desirable range, 660 µL NaOH was added into each FT while cysts were still being aerated. Subsequently, 10 mL NaOCl was added to decapsulate the cysts. Approximately 2 min was allowed until cysts colour changed to orange, after which 14 mL sodium thiosulphate ($Na_2S_2O_3$) was added to neutralise the hypochlorite. At this point, cysts were sieved over a filter of 50 µm mesh size and thoroughly rinsed with 35 g L^{-1} filtered autoclaved sea water (FASW). Rinsed decapsulated cysts of each strain were transferred into two FTs filled with FASW for hatching. The FTs were placed on a rotor (to ensure cysts were kept in continuous suspension) at a rotating speed of 5 rpm in a temperature-controlled room (28 ˚C) and exposed to a constant illumination of approximately 27 $\mu Em^{-2}sec$.

The FTs were checked regularly from 16 h of incubation onwards for appearance of first instar nauplii. One FT per strain was retrieved from the rotor at the sight of adequate amounts of free swimming nauplii. Batches of small amounts of instar I were taken and rinsed with autoclaved solutions of the different salinity concentrations over a 150 µm sterile filter before the nauplii were transferred into sterile petri dishes containing medium of the same salinity. Twenty individuals each were pipetted under the binocular microscope from the respective petri dishes into triplicate autoclaved screw cap bottles containing 20 mL of the respective experimental salinities, which was adjusted to 30 mL final volume. Feeding was done once with autoclaved *Aeromonas harveyi* (LVS3) at 10^7 CFU mL^{-1} before bottles were tightly covered and placed randomly on a rotor in the temperature-controlled room as described above. LVS3 was prepared according to standard procedures (Defoirdt *et al*., 2006) and the density was determined using the following equation:

$$\text{Concentration } (CFUmL^{-1}) = [1200 \times 10^6 \times OD] \times d$$

Where: OD = Optical density; d= Dilution factor

The second batch of FTs containing the different strains was retrieved from the rotor approximately 8 h later, which is the approximate period for molting of nauplii from instar I to II (Van Stappen, 1996b). All further experimental procedures were exactly as described for instar I above. Survival of each batch was scored at the end of 48 h. This was achieved by pouring the entire content of each FT into a petri dish and counting surviving animals under the binocular microscope.

Experiment 2: The effect of low salinity on the survival of instar I and pre-adults in xenic conditions

Artemia were incubated in low salinity medium at two different ages (i.e. at 0 days representing freshly hatched nauplii and at 8 days representing the pre-adult stage) and their survival was recorded over a period of nine days. The different salinities (0, 5, 10, 20 and 32 g L^{-1}, with the highest salinity used as control) were

prepared and checked as described in the axenic test. The test was conducted xenically. Cysts were incubated and hatched following standard procedures as described by Sorgeloos *et al*. (1986). Experimental procedures were adapted from Vanhaecke *et al*. (1984). For the animals incubated into low salinity at 0 days, 50 freshly hatched nauplii for each of the different strains were pipetted under the binocular microscope into triplicates of 500 mL bottles filled to the 300 mL mark with the different experimental saline media. Animals in each bottle were immediately fed 2 mL (at a concentration of approximately 27.5 million cells mL^{-1}) fresh *Tetraselmis suecica* algae and subsequently feeding was done *ad libitum* based on visual observation. Bottles containing nauplii were placed randomly into a water bath in a temperature (28 ℃) controlled room and aeration was supplied using tubes inserted into each of the bottles. Illumination (2,000 lux) was provided using fluorescent tubes. Every third day, culture water was changed with freshly diluted water of the same salinity and survival was scored after nine days. This was done by gently pouring the entire content of the glass vessel through a 100 µm mesh filter; trapped animals were immediately rinsed into a petri dish containing freshly prepared medium of the same salinity. Culture bottles and filter were carefully checked and properly rinsed to ensure no animal was lost. Animals were then pipetted back into the bottles and were fed and returned to the water bath. As for the animals incubated at the age of 8 days, a portion from the same batch of hatched nauplii, used for the 0-day incubation treatment, was reared for eight days in 32 g L^{-1} sea water before they were inoculated into the different experimental salinities. All further procedures were exactly as described for the 0-day incubation treatment.

Experiment 3: Testing the effect of low salinity on reproductive and life traits of the different Artemia strains

Reproductive and life traits of the different *Artemia* strains were assessed at the five low salinities used in the xenic test (Experiment 2). The group of animals which were incubated in low salinity at the nauplius stage (0 days incubation age) and grown to pre-adults in the xenic survival test were pooled according to the different salinity treatments after the nine days test period, and were used for further monitoring of their reproduction. Experimental procedures were adapted according to Browne & Wanigasekera (2000). For each strain, 35 mL culture volume of each of the salinity treatments, filled into ten replicates of 50 mL total volume FTs was used. For the bisexual strains (GSL and VC), a pair of copulating individuals was inoculated into each of the FT replicates while a single individual was used for the parthenogenetic strains (BLK and TUZ). The brood pouch in the females and hooked graspers in males aided to distinguish between the two sexes in the bisexual species (Van Stappen, 1996b). After the animals were inoculated, 1 mL (at a concentration of approximately 27.5 million cells mL^{-1}) of fresh *Tetraselmis suecica* algae were added as feed into each FT; the FT were placed in water baths in a temperature controlled room at 28 ^{o}C. Subsequent feeding was done *ad libitum* based on visual observation. FTs were monitored daily for mortality and reproduction. In the case of any mortality, the dead animal was replaced with a living individual from the stock population of the same strain and salinity. For the sexual strains, dead males were replaced continually as long as the females were alive and a culture replicate was terminated once the female died. For the parthenogenetic strains, a culture replicate was terminated after the death of a reproductive female (Agh *et al*., 2008). As for reproduction, all free swimming nauplii and cysts sighted in FTs were counted. This was achieved by pouring the entire content of the FT into a petri dish and offspring were pipetted under the binocular microscope. Parent animals were returned into fresh medium of the same salinity, fed and placed back into the water bath. Data collected were used to compute: i) female pre-reproductive period (days), ii) female reproductive period (days), iii) female post-reproductive period (days), iv) total lifespan of reproductive females (days), v) offspring as cysts, vi) offspring as nauplii, vii) offspring per day as cysts, viii) offspring per day as nauplii, ix) total offspring per female, x) total offspring per female per day, xi) number of broods per female, xii) offspring per brood, xiii) inter brood interval (days) and xiv) percent offspring encysted.

Statistical Analysis

Analyses were performed using the SAS (SAS for Windows version 9.3) software. Data were arcsine transformed and the assumptions of normality and homoscedasticity were checked. The effects of three factors in Experiment 1 and 2 (salinity, strain and developmental stage at incubation) were included in a full model (ANOVA) analysis with their interactions. The dependent variable in both tests was the survival of the animals. Where interaction existed, one-way analysis of variance (ANOVA) between one main effect at each level of a second main effect was

performed. For the reproductive and life traits ANOVA was employed to determine the effect of salinity and strain, respectively, on the reproductive parameters tested. For all the respective tests, where heteroscedasticity was observed, Welch's ANOVA was performed instead. Where ANOVA results indicated significance between the treatments, Bonferroni post-hoc test was performed to compare and identify differences between the means. P-values of less than 0.05 were considered significant. As 100 % mortality was recorded at 0 g L^{-1} salinity for all the strains tested in Experiment 1 and 2, it was excluded from the analyses.

RESULTS

Effect of low salinity on the survival of instar I and II developmental stages of the different strains in axenic condition

Generally, a high survival of the different strains used was observed. And highly significant interaction existed among the three factors assessed (salinity, strain and developmental stage) (F = 5.12, P = 0.0001). Salinity contributed most to the explanation of the result; hence, the data were sorted by salinity. When the interaction effect was removed and the data were further analysed using the one-way ANOVA, the results revealed that salinity had significant effect on survival with respect to the strain factor, whereas on the contrary, salinity had no significant effect on survival with respect to the instar developmental stage (Table 2). The post-hoc analysis showed that at 5 g L^{-1}, the BLK strain had significantly higher survival (96.7 ± 2.6 %; p<0.05) than the TUZ strain (71.7 ± 25.8 %), whereas the two sexual strains showed intermediate survival, not significantly different from either BLK or TUZ (Figure 1). At 32 g L^{-1}, it was the TUZ strain which had significantly higher survival (100.0 ± 0.0 %) than the BLK strain (94.2 ± 3.8 %), with again the sexual strains in-between. No significant difference (p>0.05) was observed between strains at 10 g L^{-1} (Figure 1) with minimum survival of 95.0 ± 3.2 % for the TUZ strain and highest survival 99.2 ± 0.8 % for the BLK strain. Differences in survival between the two instar stages were not significant (p > 0.05) at each salinity concentration (Figure 2).

Effect of low salinity on the survival of instar I and pre-adults in xenic conditions

Similar to the axenic test, high survival rate was also observed at most of the salinities tested during the xenic test, except at 5 g L^{-1}. Highly Significant interactions also occurred between salinity, strain and incubation age (F = 13.53; P = 0.0001). Notably, salinity had significant effect on survival with respect to other factors: strain and incubation age (Table 3). Differences in survival were observed between strains at 20 and 32 g L^{-1} (P < 0.05), but not at 5 and 10 g L^{-1} (Figure 3). Highest survival at 20 g L^{-1} (97.8 ± 2.1 %) and at 32 g L^{-1} (98.0 ± 2.0 %) was observed with the GSL strain, while the lowest survival (87.4 ± 10.4 %; 57.4 ± 35.9 % respectively), was observed with the BLK strain. At these two salinities GSL values were significantly higher (P < 0.05) than both TUZ and BLK values. Differences between both incubation ages were observed only at 5 g L^{-1} (Figure 4). The highest survival (93.6 ± 4.6 %) was observed when the animals were inoculated at 8 days at 20 g L^{-1}; however, this did not significantly differ from survival when inoculated at 0 days at the same salinity (P>0.05). This pattern was also observed at 10 and 32 g L^{-1} salinities where survival was also high.

Effect of low salinity on reproductive and life traits of the different Artemia strains

Due to total mortality of the animals at 0 g L^{-1} salinity during the xenic test (Experiment 2), only 5, 10, 20 and 32 g L^{-1} salinity were included in experiment 3. Mortality also persisted among the two parthenogenetic strains at 5 g L^{-1}, whereby no animal reproduced. Furthermore, although these two strains were observed to reproduce at 10, 20 and 32 g L^{-1}, neither had all animals reproducing in any of the salinities. As for the sexual strains, reproduction occurred at all the salinities tested, including the lowest 5 g L^{-1}. Generally, the two sexual strains had a higher number of reproducing females than the parthenogenetic strains. The lowest percentage (20 and 30 %) of females that reproduced was recorded with the BLK and TUZ strains, respectively, at 10 g L^{-1} salinity. On the other hand, the highest percentage (100 %) of females that reproduced was observed with GSL at 20 and 32 g L^{-1} and with VC at 32 g L^{-1} salinity.

Salinity and strain had a significant effect on a number of reproductive and life traits tested. Out of the fourteen traits considered, salinity had a significant effect (P < 0.05) on five (Table 4), whereas the strain factor had a significant effect (P < 0.05) on 11 traits (Table 5). Salinity and strain each had a significant effect on three among the traits, i.e. female reproductive period, total lifespan of reproductive

Table 2: ANOVA results performed for the survival of four *Artemia* strains and two instar developmental stages in three salinities during the 48 h axenic test

Salinity (gL^{-1})	Strain		Instar developmental stage	
	F-ratio	P-value	F-ratio	P-value
5	4.04	0.021	0.64	0.433
10	1.73	0.193	3.36	0.081
32	3.62	0.031	0.000	1.00

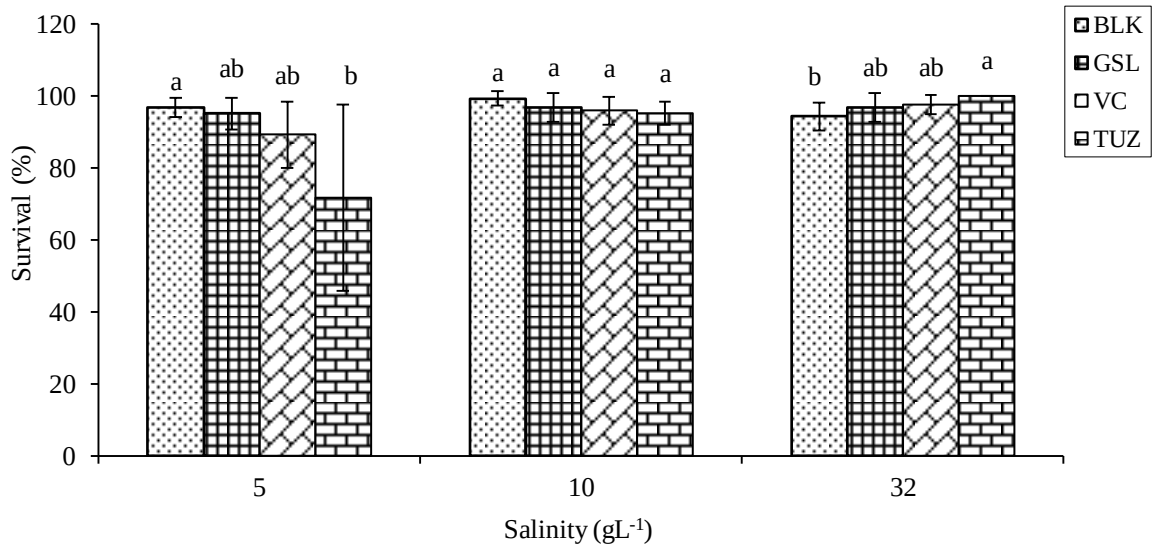

Figure 1: Survival of four *Artemia* strains cultured at different low salinities during a 48 h axenic test. (Different superscripts show significant difference (p<0.05) between means ± standard deviation per salinity)

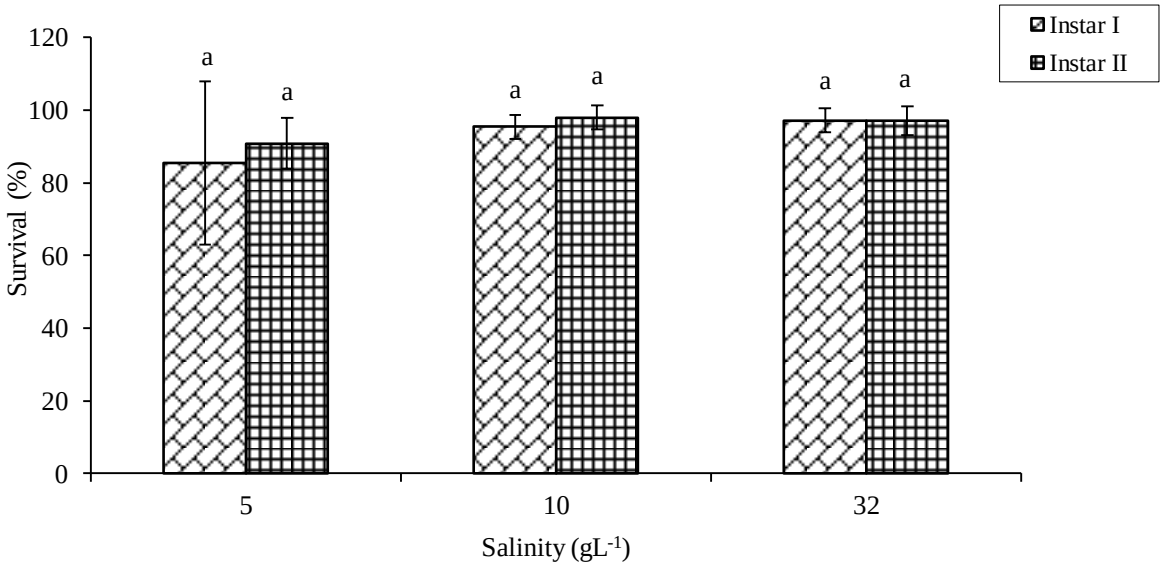

Figure 2: **S**urvival at instar I and II developmental stages of four *Artemia* strains cultured at different low salinities during a 48 h axenic test. (Different superscripts show significant difference (p<0.05) between means ± standard deviation per salinity)

Table 3: ANOVA results performed for survival of four *Artemia* strains and two incubation ages in four salinities during a nine day xenic test

Salinity (g L^{-1})	Strain		Inoculation age	
	F-ratio	P-value	F-ratio	P-value
5	2.67	0.054	26.96	0.0001
10	2.89	0.052	0.17	0.685
20	4.40	0.007	2.56	0.114
32	10.44	0.0001	0.23	0.636

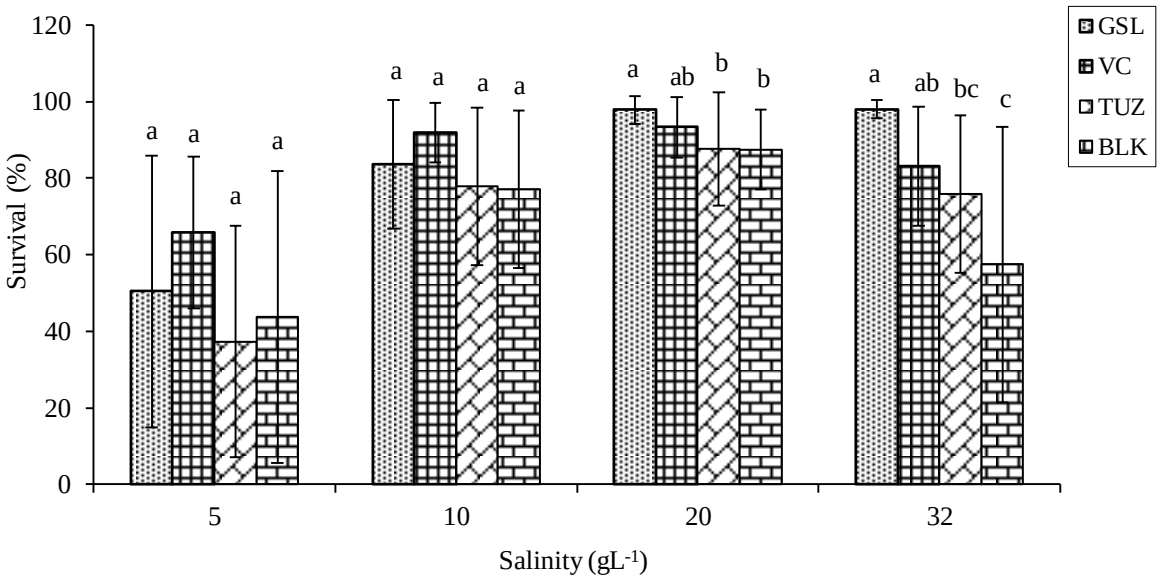

Figure 3: Survival of four *Artemia* strains cultured at different low salinities during a nine day xenic test. (Different superscripts show significant difference (p<0.05) between means ± standard deviation per salinity)

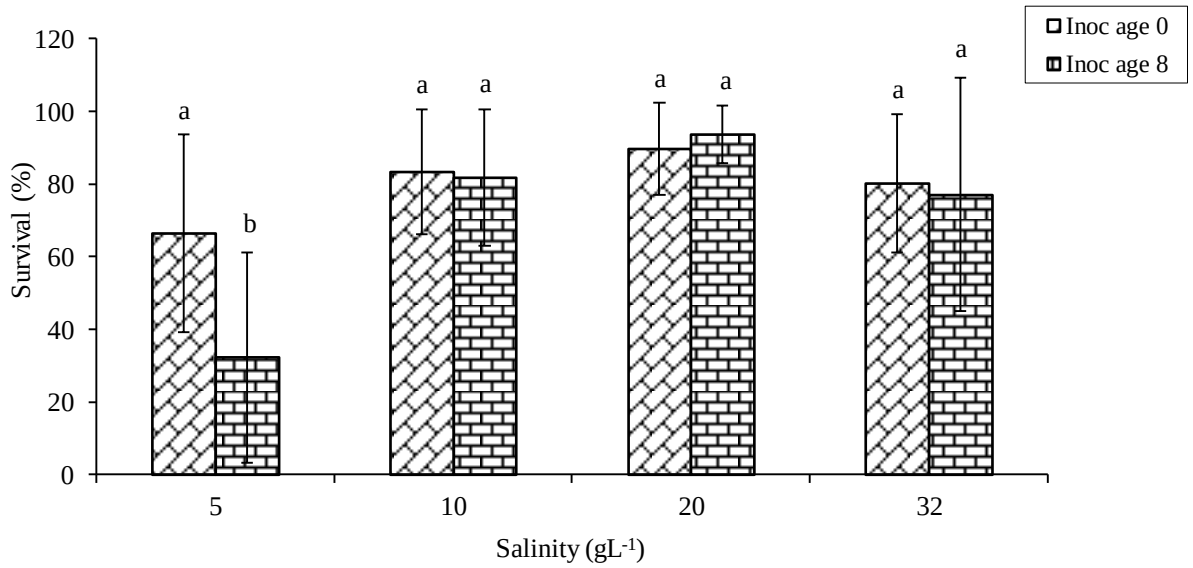

Figure 4: **S**urvival at inoculation age 0 and 8 days of four *Artemia* strains cultured at different low salinity during a nine day xenic test. (Different superscripts show significant difference (p<0.05) between means ± standard deviation per salinity).

Table 4: Effects of different salinity levels on the reproductive and life traits of the different Artemia strains.

Reproductive and Life Traits	Salinity (g L^{-1})			
	5	10	20	32
Female reproductive period*	0.70±2.4^{b}	1.2±2.2^{b}	4.4±5.1^{a}	4.4±3.8^{a}
Parameters	1.0±1.2^{b}	3.5±3.4^{ab}	4.7±4.1^{a}	2.4±1.8^{b}
Total lifespan of reproductive females*	20.3±4.1^{b}	24.7±4.1^{ab}	27.9±8.1^{a}	23.7±3.3^{b}
Total offspring per female	56.6±101.6^{b}	110.3±95.9^{ab}	174.5±203.2^{ab}	214.4±203.2^{a}
Interbrood interval	0.3±1.1^{ab}	0.2±1.2^{b}	2.0±3.3^{ab}	2.7±2.9^{a}

Values represent mean ± standard deviation (n = 10). Different superscripts show significance between means in rows. Traits with significant differences are shown. * = In days

Table 5: Effect of strain types on the reproductive and life traits of *Artemia*

Parameters	Strain			
	BLK	TUZ	VC	GSL
Female pre-reproductive period*	23.1±4.9^{a}	19.5±2.9^{a}	15.6±4.2^{b}	13.5±3.9^{b}
Female reproductive period*	0.5±1.5^{c}	1.2±3.3^{bc}	3.5±3.8^{b}	5.5±4.6^{a}
Total lifespan of reproductive female*	28.1±11.6^{a}	23.8±4.0^{ab}	22.5±3.1^{b}	22.2±4.5^{b}
Offspring as cysts	16.1±30.8^{b}	6.4±29.2^{b}	104.5±119.4^{a}	67.0±63.0^{ab}
Offspring as nauplii	12.5±69.4^{b}	41.4±52.8^{b}	115.5±198.2^{ab}	200.5±191.8^{a}
Offspring per day as cysts	26.2±29.4^{ab}	10.2±24.5^{b}	61.3±67.5^{a}	21.72±41.7^{b}
Offspring per day as nauplii	40.4±48.2^{ab}	66.3±38.5^{a}	23.1±28.6^{b}	62.5±53.1^{a}
Total offspring per female	36.6±68.9^{b}	58.2±42.5^{b}	188.9±179.9^{a}	272.1±207.0^{a}
Broods per female	1.0±0.5^{b}	1.0±0.4^{b}	1.7±1.0^{ab}	2.0±1.3^{a}
Offspring per brood	50.4±31.6^{b}	71.9±21.3^{b}	116.9±64.9^{a}	135.8±64.9^{a}
Percent offspring encysted	49.3±43.0^{ab}	22.4±35.6^{b}	66.1±41.8^{a}	26.1±23.1^{b}

Values represents mean ± standard deviation (n = 10). Different superscripts show significance between means in rows
TUZ = Tuz strain, BLK = Balikun strain, GSL = Great Salt Lake strain, VC = Vinh Chau strain.
Traits with significant differences are shown. * = In days

females and total offspring ($P < 0.05$). Analysis for strain effect on female pre-reproductive period revealed that the GSL *A. franciscana* started reproduction significantly earlier at 13.5 ± 3.9 days compared to the parthenogenetic strains. Generally, more reproductive days were observed as salinity increased. A significantly longer reproductive period (4.4 days) was recorded at 20 and 32 g L^{-1} than at 5 and 10 g L^{-1} with 0.7 and 1.2 days respectively. BLK had the shortest period of reproduction (0.5 ± 1.5 days), while GSL reproduced significantly in more days (5.5 ± 4.6; $P < 0.05$).

The two parthenogenetic strains BLK and TUZ had a longer total lifespan of 28.1 ± 11.6 and 23.8 ± 4.0 days respectively than the sexual ones. BLK was significant different from the sexual ($P < 0.05$) with to total lifespan whereas no significant difference was found between the two parthenogenetic strains. Similarly, no significant difference was observed in the total lifespan of the two sexual strains VC (22.5 ± 3.1 days) and GSL (22.2 ± 4.5 days). With respect to the effect of salinity, significantly longer total lifespan (27.9 ± 8.1 days) was observed at 20 g L^{-1} than at 5 and 32 gL^{-1} however the difference with 10 g L^{-1} was not significant. GSL produced significantly more nauplii

per female (200.5 ± 191.8) than the two parthenogenetic strains ($P<0.05$) (the difference with VC was not significant). The BLK strain had the lowest number of nauplii (12.5 ± 69.4) produced per female. The percentage offspring produced as cysts was the lowest in GSL and TUZ strains at 26.1 ± 23.1 and 22.4 ± 35.6 percent respectively.

DISCUSSION

In nature, *Artemia* are inhabitants of varying saline environments. Different populations are adapted to certain specific salinity conditions, whereby some strains are found in environments with very high salinity (Gajardo & Beardmore, 2012). Nevertheless, when salinity tends towards high levels, *Artemia* becomes physiologically stressed (Van Stappen, 2008). This could affect normal body functions such as reproduction and may ultimately lead to mortality. On the other hand, it is known that this organism thrives well at sea water salinity (Van Stappen, 1996a). In the present study, it was hypothesized that low salinity levels could support good physiological functions in the animals which may result in high survival and ovoviviparity. Ovoviviparity in *Artemia* has always been considered to occur only when conditions are favourable and particularly at relatively lower salinity. Hence, different low salinity levels (0 - 32 g L^{-1}) were used to test the survival, reproductive and life traits of four *Artemia* strains (Great Salt Lake and Vinh Chau strains of *Artemia franciscana*; Tuz and Balikun strains of parthenogenetic *Artemia*). However, it has been reported that the instar I (*Artemia* nauplius) stage is more tolerant to high salinity shocks than later stages (Vanhaecke & Sorgeloos, 1980), but it has never been tested if this is also the case for low salinity shocks. Therefore, the performance of instar II and pre-adult *Artemia* was tested, after incubation in lower salinity, in comparison with instar I nauplii. Four different strains were used as it was assumed that low salinity tolerance might be strain-specific. Additionally, the reproductive and life traits of the strains used in the study were also assessed at the different low salinities. The aim of the study was to select the most suitable low salinity level and strain, as well as to obtain information on the most appropriate developmental stage for the purpose of inoculation during mass culture for continuous ovoviviparous nauplii production.

The selection of the strains for the test was based on their availability on the global cyst market and their stress tolerance. The Great Salt Lake is known to be a native North American strain of *Artemia franciscana* (Lavens & Sorgeloos, 2000). *Artemia franciscana* has an invasive ability; as a result, intentional and unintentional inoculations have been carried out related with aquaculture activities (Ruiz *et al*., 2008). The Vinh Chau strain of *Artemia franciscana*, whose origin is from the San Francisco Bay strain, is an example of such inoculations into Vietnam (Kappas *et al*., 2004). Investigations from multidisciplinary perspective, using reproductive characteristics, allozyme and mitochondrial DNA analyses, have shown an evident divergence between the SFB and VC strains, with the VC strain showing high tolerance to stress such as for example induced by temperature (Kappas *et al*., 2004). Cysts of *Artemia franciscana* from the coastal saltworks in San Francisco Bay, California, USA and from an inland biotope, the Great Salt Lake, Utah, USA have been exported worldwide since 1950 making them of high commercial relevance (Lavens & Sorgeloos, 2000). As for the two parthenogenetic strains, they are also harvested, but of more limited commercial relevance. They were included because according to Browne & Wanigasekera (2000), parthenogenetic strains would be better adapted to extreme environmental conditions.

In a first experiment, the performance of instar I and instar II *Artemia* was compared in axenic conditions, which is also referred to as the gnotobiotic *Artemia* culture (GART) system (Baruah *et al*., 2010). The GART system was developed for the study of host-microbe interactions and offers a unique way of eliminating the interference of all microbial communities that are naturally present in the rearing environment. Since the focus in the present study was to test the effect exclusively of salinity on the survival of the animals, the system was adopted in order to eliminate all such unknown microbial communities, which the test animals may feed on, hence increasing their tolerance and altering the effect of the salinity itself. Although the axenic test lasted for only 48 h, the absence of any form of bacteria in the culture media [apart from the autoclaved *Aeromonas harveyi* (LVS3) used as feed], allowed for a good insight on the effect of salinity and the other factors (i.e. strain and instar developmental stages) on survival. The observed high survival (≥90 %) at salinities from 5 – 32 g L^{-1}, suggests that this range of low salinity is highly supportive of the animals' performance within the culture period. The relatively lower survival (71.7 %) observed with the TUZ strain at 5 g L^{-1} could be indicating strain preference to different salinities. This was particularly observed with the two parthenogenetic strains. While it appears that the BLK strain may be better adapted to the lowest salinity tested, the TUZ

strain on the other hand seems to prefer the highest salinity tested (Figure 1). The two bisexual strains on the other hand performed in a similar way at all the salinities tested. Salinity tolerance of *Artemia* is related to the activity of the Na, K-ATPase enzyme. With reference to the activity of this enzyme in the nauplii of *Artemia*, Lee & Watts (1994) showed increases of this enzyme in emerged embryos and during the first instar stage. Since the maintenance of balance of the enzyme in these developmental stages of the nauplii is by nature programmed to occur independent of the environmental salinity, it must be supportive of good performance such as survival in the animals. Although the Na, K-ATPase enzyme activity was not investigated in the present study, the non-significant difference observed between the survival rates of the two instar developmental stages of all the strains at the respective salinities tested (Figure 2) may suggest that the processes for the activity of the enzyme in instar II could be similar to that in instar I which is independent of the environmental salinity.

The first experiment was run in gnotobiotic conditions in order to exclude the effect of the microbial environment when testing for salinity tolerance of *Artemia*. In mass production of *Artemia*, however, the xenic approach is practiced, due to the increased size of the system and the longer culture duration compared to laboratory tests, which are mostly characterized by their small size and shorter duration. In practice, a continuous *Artemia* nauplii production system, which is supposed to run as an alternative to nauplii production through cyst hatching, would be operated anyhow in xenic conditions. Such xenic systems are associated with microbial organisms of which some are pathogenic and could constitute huge challenges leading to mortality. Other microbial organisms on the other hand can have a beneficial effect, for example as they contribute to *Artemia* nutrition in the culture (Pablo & Jones, 1993; Toi *et al*., 2013). Maintaining favourable environmental conditions such as salinity levels which support efficient osmoregulatory processes within such systems could contribute to reduced physiological stress, and may result in increased survival rate. In the present xenic experiment, in which we tested for performance of the pre-adult stage at low salinity, in comparison to instar I nauplii, the high survival recorded at the salinity range from 10 to 32 g L^{-1} indicated that the animals performed well throughout this salinity range. But at salinity lower than 10 g L^{-1}, survival was reduced significantly. Irrespective of the strain, the relatively consistent higher survival of the animals at 20 g L^{-1} suggests that this salinity was suitable for the different strains. The significantly ($P < 0.05$) higher survival rate (97.8 ± 2.1 %) (Figure 3) obtained with the GSL strain further indicated that the strain may be relatively more adapted to this level of low salinity. As in the test under axenic condition (Figure 1), a similar pattern also occurred in the xenic condition (Figure 3), whereby the BLK parthenogenetic strain appeared not so much affected by lower salinities as TUZ strain was affected. This supports the assertion that parthenogenetic strains of *Artemia* may be niche specialists (Browne & Wanigasekera, 2000).

Although *Artemia* adults and juveniles are known to be more tolerant to different environmental factors than younger stages (Wear & Haslett, 1986; Wear *et al*., 1986), they are influenced by their ambient salinity (Holliday *et al*., 1990). Comparing the effect of incubation into low salinity on freshly hatched *Artemia* nauplii and on 8 days-old pre-adults, the results indicated that the pre-adult *Artemia* could experience a shock when transferred from a higher to a relatively lower salinity medium. But this observation applies only to the lowest salinity tested (5 g L^{-1}) where the difference between the two age groups was significant. Since no significant difference was observed in survival between the two age groups at salinities in the range 10 – 32 g L^{-1}, it suggests that when using these latter salinities for mass culture purposes, it may not be needed to rear the animals to the pre-adult stage before they are incubated at lower salinity. Hence, it was concluded that using nauplii rather than pre-adults for the purpose of incubation at lower salinity would result in a more efficient use of time and other resources required in the culture of the animals.

For a successful mass culture, it is not only important that the animals survive at lower salinity, but also that they show a sufficiently high fecundity throughout their reproductive life, and that they reproduce mainly ovoviviparously. This was assessed in Experiment 3. Only the sexual strains completed their lifecycle in all the salinities where survival was recorded. On the contrary, the parthenogenetic strains could only reproduce at 10 g L^{-1} salinity and above. A similar observation was reported in a study with four sexual *Artemia* species and a parthenogenetic strain from Margherita diSavoia (Italy), where the parthenogenetic strain reproduced only in two out of nine salinity and temperature combinations (Browne & Wanigasekera, 2000). The present observation again supports the description of the parthenogenetic strains as niche specialists. The higher number of reproductive females of the sexual strains in some of the salinities tested distinguishes them from the parthenogenetic

strains in the present study. Noteworthy is that the GSL and VC strains had all (100 %) females reproducing at 20 and 32 g L^{-1}, respectively. For most of the traits assessed, the best performances were recorded at 20 g L^{-1} salinity. Since the overall objective is to achieve ovoviviparity during mass culture, only reproductive traits which are more relevant for this purpose are discussed here.

The importance of longer reproductive periods cannot be over-emphasized. More reproductive days observed in the GSL strain and at 20 g L^{-1} salinity may have contributed to the higher number of total offspring recorded for this strain. However, the number of days ranging 4.2 – 14 recorded in the present study falls short of the 23.2 days recorded by Soniraj (2004) with the Tuticorin strain of *Artemia franciscana* at 20 g L^{-1} salinity. Agh *et al*. (2008) reported 28.4, 18.0 and 20.8 reproductive days for a parthenogenetic strain from Urmia Lake, Iran, a parthenogenetic strain from lagoons around Urmia Lake and the sexual *Artemia urmiana*, respectively, but at 75 g L^{-1} salinity. Comparing results in the present study with that of Soniraj (2004) considering that the Tuticorin strain belongs to the same species with the GSL strain, and that it was also tested at 20 g L^{-1}, the difference observed may be due to differences in adaptation of the animals to specific local environmental conditions, or to differences in other experimental conditions.

Generally, the ovoviviparity observed in all salinities, at which the parent animals survived, also suggests a physiological response by the animals in relation to the salinity tested. It was clear that more nauplii (200.5 ± 191.8) were produced by the GSL strain of *Artemia franciscana*. This is high compared to the 126.7 total nauplii production per female throughout her lifetime, reported by Soniraj (2004) at 20 g L^{-1} salinity using the Tuticorin strain. However, the parthenogenetic strain used in their study produced a far higher (1,041.67) number of nauplii at the same salinity. With respect to the total offspring produced per female, the GSL strain showed a higher fecundity, though not significantly different, than the VC strain. The significantly higher total number of nauplii produced by the GSL strain, as compared with the other strains that were used, coupled with the high number of nauplii (62.5) per day indicated that the GSL strain is a better candidate for use in mass culture.

Based on results of the axenic test, it could be concluded that low salinity within the range tested, with exception of 0 g L^{-1} culture medium, supported high survival within 48 h of incubation for any of the two instar stages tested. The nine-day xenic test also indicated that a high level of survival can be expected at a salinity as low as 10 g L^{-1}, but higher survival response at 20 g L^{-1} allows for the conclusion that this low salinity level is the best relative to the others tested. With regard to the appropriate age for inoculation into low salinity, since no significant difference existed between the two inoculation ages tested at a salinity range of 10 – 32 g L^{-1}, it was concluded that to save resources such as time, energy and funds, it will be more beneficial to inoculate *Artemia* for low salinity mass culture at the nauplius stage. Considering the high survival recorded at 20 g L^{-1} salinity particularly with the GSL strain, coupled with better performance in terms of the reproductive and lifetime traits measured with this strain, it was concluded that this salinity and strain are the most suitable when considering biomass culture for the purpose of nauplii production as live food for aquaculture species.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Belgium, for supplying the cysts of the strains used in the study, and their staff, particularly Mr. Christ Mahieu and Ms Anita De Haese who provided technical assistance. We also thank Ms Gail Jordan of the Animal Sciences Department, Stellenbosch University, South Africa for the statistical analysis of the data.

REFERENCES

Aalamifar, H., Agh, N., Malekzadeh, R., & Aalinezhad, M. (2014). A comparative study on the effect of different salinities on the survival, growth, lifespan and morphometric characteristics of cysts of two parthenogenetic species of *Artemia* (GaavKhooni Wetlands of Isfahan, ponds around Lake Urmia) from Iran. *Asian Journal of Biological Science*, *7*(6), 242-251. https://doi.org/10.3923/ajbs.2014.242.251

Abatzopoulos, T. J., El-Bermewi, N., Vasdekis, C., Baxevanis A. D., & Sorgeloos, P. (2003). Effects of salinity and temperature on reproduction and lifespan characteristics of clonal *Artemia*. (International study on Artemia LXVI). *Hydrobiologia*, *492*,191-199.

Agh, N., Abatzopoulos, T. J., Van Stappen, G., Razavi Rouhani, S. M., & Sorgeloos, P. (2007). Coexistence of sexual and parthenogenetic *Artemia* populations in Lake Urmia and neighbouring lagoons. *International Review of Hydrobiology*, *92*(1), 48-60. https://doi.org/10.1002/iroh.200610909

Agh, N., Van Stappen, G., Bossier, P., Sepehri, H., Lofti, V., Razawi Rouhani, S. M., & Sorgeloos, P. (2008). Effects of salinity on survival, growth, reproductive and life span characteristics of *Artemia* populations

from Urmia Lake and neighbouring lagoons. *Pakistan Journal of Biological Sciences*, *11*(2), 164-172. https://doi.org/10.3923/pjbs.2008.164.172

Baruah, K., Ranjan, J., Sorgeloos, P., & Bossier, P. (2010). Efficacy of heterologous and homologous heat shock protein 70s as protective agents to *Artemia franciscana* challenged with *Vibrio campbellii*. *Fish and Shellfish Immunology*, *29*(5), 733-739. https://doi.org/10.1016/j.fsi.2010.07.011

Browne, R. A., & Wanigasekera, G. (2000). Combined effects of salinity and temperature on survival and reproduction of five species of *Artemia*. *Journal of Experimental Marine Biology and Ecology*, *244*(1), 29-44. https://doi.org/10.1016/S0022-0981(99)00125-2

Castro-Mejia, J., Castro-Barrera, T., Hernandez, L. H., Arredondo-Figueroa, J. L., Castro-Mejia, G., & De Lara-Andrade, R. (2011). Effects of salinity on growth and survival in five *Artemia franciscana* (Anostraca: Artemiidae) Populations from Mexico pacific coast. *Revista de Biologia Tropical*, *59*(1), 199-206. https://doi.org/10.15517/rbt.v59i1.3190

Clegg, J. S., & Trotman, C. N. A. (2002). Physiological and biochemical aspects of *Artemia* Ecology. In T. J. Abatzopoulos, J.A. Beardmore, J. S. Clegg & P. Sorgeloos (Eds), *Artemia*: *Basic and Applied Biology* (pp.129-170). Kluwer Academic.

Defoirdt, T., Crab, R., Wood, T. K., Sorgeloos, P., Verstraete, W., & Bossier, P. (2006). Quorum sensing-disrupting brominated furanones protect the gnotobiotic brine shrimp *Artemia franciscana* from pathogenic *Vibrio harveyi*, *Vibrio campbellii* and Vibrio parahaemolyticus isolates. *Applied and Environmental Microbiology*, *72*(9), 6419–6423. https://doi.org/10.1128/aem.00753-06

El-Bermawi, N., Baxevanis, A. D., Abatzopoulos, T. J., Van Stappen, G., & Sorgeloos, P. (2004). Salinity effects on survival, growth and morphometry of four Egyptian *Artemia* populations (International study on *Artemia*, LXVII). *Hydrobiologia*, *523*, 175-188. https://doi.org/10.1023/b:hydr.0000033124.49676.5c

Gajardo, G. M., & Beardmore, J. A. (2012). The brine shrimp *Artemia*: adapted to critical life conditions. *Frontiers in Physiology*, 3, Article 185. https://doi.org/10.3389/fphys.2012.00185

Holliday, C. W., Roye, D. B., & Roer, R. D. (1990). Salinity-induced changes in branchial Na^+/K^+-ATPase activity and transepithelial potential difference in the brine shrimp, *Artemia salina*. *Journal of Experimental Biology*, *151*(1), 279-296. https://doi.org/10.1242/jeb.151.1.279

Kappas, I., Abatzopoulos, T. J., Van Hoa, N., Sorgeloos, P., & Beardmore, J. A. (2004). Genetic and reproductive differentiation of *Artemia franciscana* in a new environment. *Marine Biology*, *146*(1), 103–117. https://doi.org/10.1007/s00227-004-1420-9

Lavens, P., & Sorgeloos, P. (1987). Design, operation and potential of a culture system for the continuous production of *Artemia* nauplii. *Artemia* Research and its application. In P. Sorgeloos, D. A. Bengtson, W. Decleir & E. Jasper (Eds.), *The Brine Shrimp Artemia: Ecology, culturing, use in aquaculture* (pp. 340-345). Universa press.

Lavens, P., & Sorgeloos, P. (1991). Production of *Artemia* in culture tanks. In R. A. Browne, P. Sorgeloos & C. N. A. Trotman (Eds.), *Artemia Biology* (pp. 317-350).

Lavens, P., & Sorgeloos, P. (2000). The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. *Aquaculture*, *181*(3-4), 397-403. https://doi.org/10.1016/S0044-8486(99)00233-1

Lee, K. J., & Watts, S. A. (1994). Specific activity of Na^+ K^+ ATPase is not altered in response to changing salinities during early development of the brine shrimp, *Artemia franciscana*. *Physiological Zoology*, *67*(4), 910-924. https://doi.org/10.1086/physzool.67.4.30163871

Pablo, I., & Jones, D. A. (1993). Bacteria as food for *Artemia*. *Aquaculture*, *113*(1-2), 115-127. https://doi.org/10.1016/0044-8486(93)90345-y

Ruiz, O., Amat, F., & Navarro, J. C. (2008). A comparative study of the fatty acid profile of *Artemia franciscana* and *A. persimilis* cultured at mesocosm scale. *Journal of Experimental Marine Biology and Ecology*, *354*(1), 9-16. https://doi.org/10.1016/j.jembe.2007.09.015

Soniraj, N. (2004). Effect of salinity on the life span and reproductive characteristics of brine shrimps in the salt pans in Tuticorin. *Journal of the Marine Biological Association of India*, *46*(2), 133-140.

Sorgeloos, P., Lavens, P., Le´ger, P., Tackaert, W., & Versichele, D. (1986). *Manual for the culture and use of the brine shrimp Artemia in aquaculture*. Faculty of Agriculture, State University of Ghent, Belgium.

Soundarapandian, P., & Saravanakumar, G. (2009). Effect of different salinities on the survival and growth of *Artemia* spp. *Current Research Journal of Biological Sciences*, *1*(2), 20–22.

Toi, H. T., Boeckx, P., Sorgeloos, P., Bossier, P., & Van Stappen, G. (2013). Bacteria contribute to *Artemia* nutrition in algae-limited conditions: A laboratory study. *Aquaculture*, 388–391, 1–7. https://doi.org/10.1016/j.aquaculture.2013.01.005

Van Stappen, G. (1996a). Use of cysts. In P. Lavens & P. Sorgeloos (Eds.), *Manual on the production and use of live food for aquaculture*. (No. 361, pp.107-130). FAO Fisheries Technical Paper.

Van Stappen, G. (1996b). Introduction, biology and ecology of *Artemia*. In P. Lavens & P. Sorgeloos (Eds.), *Manual on the production and use of live food for aquaculture*. (No. 361, pp.79-106) FAO Fisheries Technical Paper.

Van Stappen, G. (2002). Zoogeography. In T. J. Abatzopoulos, J.A. Breardmore, J. S. Clegg & P. Sorgeloos (Eds), *Artemia basic and applied biology* (pp. 171-215) Kluwer Academic Publishers. https://doi.org/10.1007/978-94-017-0791-6

Van Stappen, G. (2008). *Artemia biodiversity in Central and Eastern Asia*. (Doctoral Thesis, Ghent University) Belgium.

Vanhaecke, P., Siddall, S. E., & Sorgeloos, P. (1984). International study on *Artemia*. XXXII. Combined

effects of temperature and salinity on the survival of *Artemia* of various geographical origins. *Journal of Experimental Marine Biology and Ecology*, *80*(3), 259-275. https://doi.org/10.1016/0022-0981(84)90154-0

Vanhaecke, P., & Sorgeloos, P. (1980). International study on *Artemia*. XIV. Growth and survival of *Artemia* larvae of different geographical origin in a standard culture test. *Marine Ecology Progress Series*, *3*, 303-307.

Wear, R. G., & Haslett, S. J. (1986). Effects of temperature and salinity on the biology of *Artemia franciscana* (Kellogg) from Lake Grasmere New Zealand. 1. Growth and mortality. *Journal of Experimental Marine Biology and Ecology*, *98*, 153-166. https://doi.org/10.1016/0022-0981(86)90080-8

Wear, R. G., Haslett, S. J., & Alexander, N. K. (1986). Effects of temperature and salinity on the biology of *Artemia franciscana* (Kellogg) from Lake Grasmere, New Zealand 2. Maturation, fecundity and generation times. *Journal of Experimental Marine Biology and Ecology*, *98*,167-183. https://doi.org/10.1016/0022-0981(86)90081-x

Wedemeyer, G. A. (1996). *Physiology of fish in intensive culture system* (1st ed.). Chapman and Hall. https://doi.org/10.1007/978-1-4615-6011-1

Conflicts of Interest: The authors declare that no conflicts of interest exist in respect to publishing these research findings.

Citation: Bwala, R. L., Salie, K., & Van Stappen, G. (2021). Survival and reproductive responses of four *Artemia* strains in low salinity. *West African Journal of Fisheries and Aquatic Sciences*, *2*(1), 9-21.

Article Link:
http://journals.unilag.edu.ng/index.php/wajfas/article/view/1276