Received: 18 July 2023 | Revised: 26 March 2024 | Accepted: 1 April 2024

DOI: 10.1002/aff2.166

AQUACULTURE, FISH and FISHERIES Open Access

WILEY

SHORT COMMUNICATION

Salinity tolerance, growth and survival of three *Artemia franciscana* (Kellogg, 1906) populations under laboratory conditions

Morine M. Ngarari[1,3] | **Sheban M. Hinzano**[1] | **Mary A. Opiyo**[2] | **Derrick G. Rugendo**[1] | **David O. Midumbi**[1] | **Francis A. Okalo**[1] | **Betty M. Nyonje**[1] | **Charles C. Ngugi**[3] | **Charles W. Gatune**[3]

[1]Mombasa Research Centre, Kenya Marine and Fisheries Research Institute, Mombasa, Kenya

[2]National Aquaculture Research Centre, Kenya Marine and Fisheries Research Institute, Sagana, Kenya

[3]Karatina University, Karatina, Kenya

Correspondence
Morine M. Ngarari, Kenya Marine and Fisheries Research Institute, Mombasa Research Centre, P.O. Box 81651-80100, Mombasa, Kenya.
Email: morinemukamik@gmail.com

Funding information
Western Indian Ocean Marine Science Association, Grant/Award Number: MASMA/OP/2020/02

Abstract

In the 1980s, *Artemia franciscana* from San Francisco Bay (SFB) was introduced into Kenyan saltworks, where it has colonized and established stable populations. However, little is known about its biology, particularly with respect to its parental SFB population. This study compared the salinity tolerances of Kenyan (KEN) population, their SFB progenitors and those of Great Salt Lake (GSL) populations. Growth and survival of these *A. franciscana* populations were evaluated under varying salinity levels in a laboratory set up. *A. franciscana* nauplii were cultured at a rate of 1 nauplii/mL in 36 Erlenmeyer flasks and fed microalgae (*Chaetoceros* sp.) at 1.5×10^6 cells/animal/day for 8 days. Survival was evaluated daily and survivors were fixed in individual vials with Lugol solution. The total length of each fixed *A. franciscana* nauplii *specimen* was measured under a compound microscope. All populations were susceptible to salinities greater than 100 g/L. Compared with the parental SFB population, the KEN population exhibited significantly reduced survival and growth at 140 g/L, suggesting a narrower salinity tolerance range. These findings underscore the need for further studies focusing on other physiological parameters, abiotic factors and genetic characterization to confirm whether the KEN population is experiencing ecological adaptation. This will contribute to the optimization of *Artemia* practices in various salinity environments as a result of climate change.

KEYWORDS
A. franciscana, growth, Kenya, salinity, survival

1 | INTRODUCTION

Artemia franciscana, a species of branchiopod crustacean, is adapted to high-salinity environments (Vanhaecke et al., 1987). The switch between dormant cysts and live-bearing cysts can occur depending on the environmental conditions. *Artemia* plays a pivotal role in the larviculture of marine fish, crustaceans, freshwater and ornamental species; thus, *Artemia* cyst production has attracted increased interest (Bahr et al., 2021; Veeramani et al., 2018). The establishment of *Artemia* resources has been strongly linked to success in the development of shrimp hatcheries. The ability to store *Artemia* cysts for several years and rapid hatching within 24 h (Manfra et al., 2012) render them more preferred live feeds for larval rearing.

The primary sources of *Artemia* cysts are natural saline lakes and inland salt lakes. However, escalating demand coupled with the impacts of climate change has led to a noticeable shortfall in *Artemia* supply from these natural reserves. To counteract this shortage, artificial *Artemia* supply resources have been established in saltwork ponds, particularly in countries lacking natural *Artemia* resources (Food and Agriculture Organization [FAO], 2022a; van Stappen, 2002). A diverse range of *Artemia* biotopes exist globally with varying ecological characteristics, primarily differentiated by physicochemical and nutrient inputs. These environmental factors significantly influence the eco-physiological traits of inhabited *Artemia* populations (Vanhaecke & Sorgeloos, 1982). Consequently, numerous studies have been undertaken to explore the potential variability and adaptive mechanisms of *Artemia* populations across diverse biotopes worldwide.

Salinity has a significant influence on the lifespan characteristics of parthenogenetic *Artemia* (Aalamifar et al., 2014). Varó et al. (1993), stated that variations in either temperature or salinity affect the oxygen consumption of bisexual and parthenogenetic *Artemia* nauplii under hypoxic conditions. Despite *Artemia*'s extraordinary tolerance to high salinities, salinity remains the critical limiting factor in controlling *Artemia* population density both in situ and in the laboratory (Agh et al., 2008). A study by Castro-Mejía et al. (2011) revealed significant performance variations among different *Artemia* populations across varying salinity treatments with performance increasing with increasing salinity.

Despite extensive research on the impact of salinity on *Artemia* populations globally, there remains a gap in knowledge about the Kenyan introduced *Artemia* population (KEN). This study aimed to compare the salinity tolerance of KEN against well-documented populations from San Francisco Bay (SFB) and Great Salt Lake (GSL), USA. The results of this study will inform the development of effective *Artemia* culture and management strategies in Kenya, contributing to the sustainability of aquaculture operations and potentially other similar environments worldwide.

2 | MATERIALS AND METHODS

2.1 | Experimental design and setup

The study was conducted at the *Artemia* Research Laboratory of Kenya Marine and Fisheries Research Institute, Mombasa Centre, located between S4°03′19.29″ and E39°40′54.53″. A 3 × 4 factorial randomized experimental design was employed comprising three *A. franciscana* populations (SFB, GSL and KEN) and four salinity treatments (35 g/L as a control, 70, 105 and 140 g/L). Each combination of population and salinity was tested in triplicate, yielding 36 experimental units.

2.2 | *Artemia* cyst preparation and hatching

Two hundred micrograms of cysts from each population were decapsulated and incubated for 18 h in 35 g/L saline water according to Lavens et al. (1996). Hatched instar I *A. franciscana* nauplii were collected using a 100 μm sieve, rinsed with running water for 5 min and concentrated in a 100 mL beaker for each population.

2.3 | Culturing conditions

Thirty newly hatched nauplii were transferred into 50 mL Falcon tubes with 30 mL of culture water to achieve a stocking density of 1 nauplii/mL. The nauplii were fed daily with microalgae (*Chaetoceros* sp.) at 1.5 $\times 10^6$ cells/animal/day for 8 days as recommended by Naegel (1999). Survival was monitored daily by transferring the contents of each Falcon tube to a Petri dish, rinsing the tube and returning live *Artemia* to the original saline water in the Falcon tube while counting.

2.4 | Data collection and analysis

Initial and final growth measurements of *Artemia* were collected for growth assessment. An initial sample of 15 nauplii from each treatment was fixed in 1% Lugol solution for subsequent length measurements at the beginning and end of the experimental period. The growth and survival data were arcsine transformed as per standard statistical procedures. Two-way analysis of variance (ANOVA) was performed to compare growth and survival rates across the treatments, utilizing Tukey's pairwise multiple comparison test to identify significant differences. Multiple regression analysis was used to explore the relationships between salinity levels and the survival and growth outcomes of each *Artemia* population. Statistical significance was established at the 95% confidence level. All analyses were conducted using R-Statistical Software (version R 4.2.3).

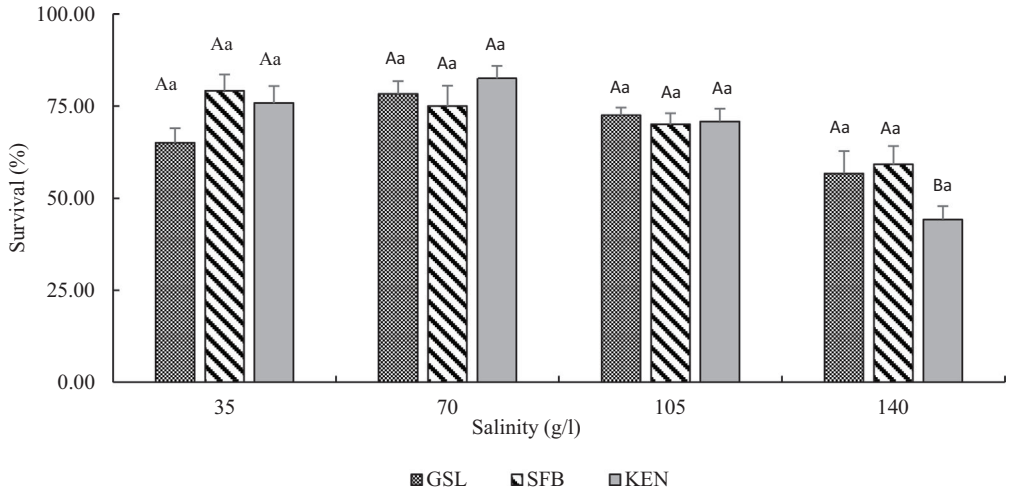

FIGURE 1 Survival rates of the three *Artemia* populations at different salinity treatments. Error bars indicate ± standard error. Upper case letters compare performance of an individual population across different salinity levels. Lower case letters compare performance of different populations within the same salinity level. Different letters indicate significance difference between treatments.

3 | RESULTS

3.1 | Survival

The percentage survival of the *A. franciscana* populations did not significantly differ among the salinity treatments of 35, 70 and 105 g/L ($p > 0.05$). At the highest salinity level tested (140 g/L), the survival of SFB and GSL did not significantly deviate from that observed at lower salinities ($p > 0.05$). However, KEN exhibited a significant reduction in survival at 140 g/L salinity compared to that at lower salinities ($p < 0.05$) with its highest survival being observed at 70 g/L salinity. Two-way ANOVA indicated a significant interaction effect between salinity levels and population types on survival rates ($p < 0.05$) indicating different responses to increased salinity among populations (Figure 1).

3.2 | Growth performance

Growth measured as the increase in the total length of *Artemia* nauplii varied significantly across the different salinity treatments ($p < 0.05$) with the highest growth recorded at 35 g/L and the lowest at 140 g/L for all populations. Decreased growth was observed at 70 g/L salinity, although the growth did not significantly differ between GSL and SFB or between SFB and KEN ($p > 0.05$). However, GSL demonstrated significantly greater growth than did KEN at 140 g/L salinity ($p < 0.05$). Two-way ANOVA revealed significant effects of both population type and salinity level on growth ($p < 0.05$) but no significant interaction effect was detected ($p > 0.05$), suggesting that although growth responses varied among populations and salinity levels, the interaction between these factors was not significant (Figure 2).

3.3 | Relationship between salinity (g/L) and *Artemia* performance

Multiple regression analysis revealed a significant negative correlation between salinity levels and survival rates across all populations ($r(12) = 0.89$, $p < 0.05$ (Table 1). This indicates that increased salinity adversely affected survival. Similarly, a significant negative correlation was found between salinity levels and growth ($r(116) = 0.52$, $p < 0.05$), confirming that growth decreased with increasing salinity for all tested populations (Table 2).

4 | DISCUSSION

This study demonstrated the significant impact of salinity on the survival and growth of *A. franciscana* populations, which is similar to the findings of Mali et al. (2023). Our findings revealed that while all populations exhibit tolerance to a wide range of salinities, KEN shows a distinct decline in survival and growth at higher salinity levels, particularly beyond 100 g/L. This outcome suggested a narrower salinity tolerance range for KEN compared to SFB and GSL, highlighting potential adaptive differences.

Previous studies have indicated that *Artemia* species exhibit a remarkable ability to tolerate and thrive across a broad spectrum of salinities because of their highly efficient osmoregulatory system (Aalamifar et al., 2014; Agh et al.,2008 ; El-Bermawi et al., 2004), which improves at salinities between 100 and 120 g/L (Triantaphyllidis et al., 1995; van Stappen, 2002). An increase in salinity leads to an increase in osmotic stress, low oxygen concentration, desiccation and increased metabolic rate to cope with the high energy demand for osmoregulation (Gajardo & Beardmore, 2012), all of which affect the biological parameters of *Artemia*. Lavens et al. (2007) and Rodríguez-Almaraz

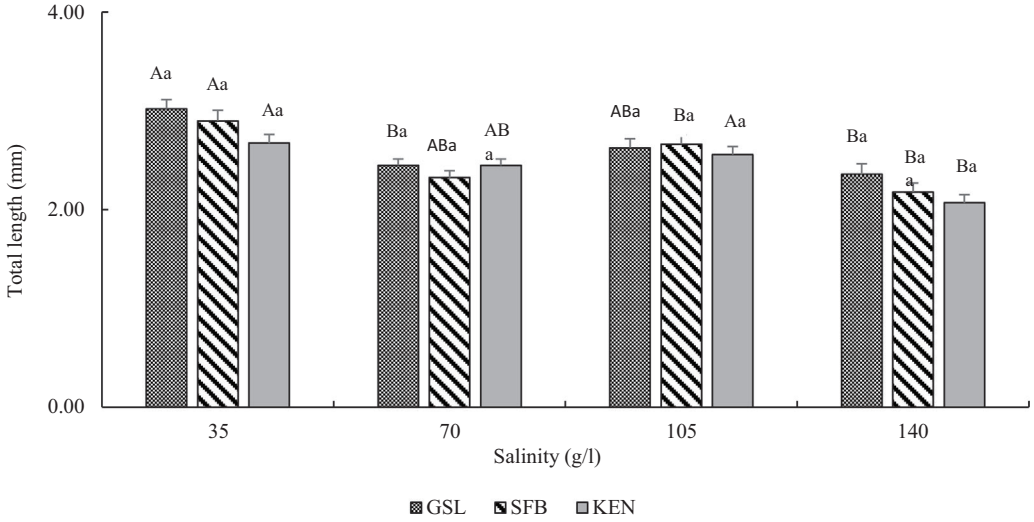

FIGURE 2 Growth performance of the three *Artemia* populations at different salinity treatments. Error bars indicate ± standard error. Upper case letters compare performance of an individual population across different salinity levels. Lower case letters compare performance of different populations within the same salinity level. Different letters indicate significance difference between treatments.

TABLE 1 Summary of multiple regression analysis between percent survival (%) and salinity (g/L).

	Coefficients	*Standard error*	*t* Stat	*p-Value*
Intercept	266.55	35.36	7.54	0.00
KEN	−1.82	0.39	−4.71	0.00
GSL	1.60	0.65	2.44	0.03
SFB	−2.30	0.59	−3.89	0.00

Abbreviations: GSL, Great Salt Lake; KEN, Kenyan; SFB, San Francisco Bay.

TABLE 2 Summary of multiple regression analysis between growth (mm) and salinity (g/L).

	Coefficients	*Standard Error*	*t* Stat	*p-Value*
Intercept	222.92	20.76	10.74	0.00
GSL	−15.60	6.07	−2.57	0.01
SFB	−15.82	5.79	−2.73	0.01
KEN	−22.49	6.70	−3.36	0.00

Abbreviations: GSL, Great Salt Lake; KEN, Kenyan; SFB, San Francisco Bay.

et al. (2006) mentioned an ecological response imprinted in the genome of *Artemia* that allows them to colonize higher salinities to avoid predation at lower salinities. The superior genetic make-up of SFB has allowed it to survive in extreme environments (Amat et al., 2005; FAO, 2022b; Gajardo & Beardmore, 2012). As KEN came from SFB, a similar performance to that of SFB was expected. According to Lahti et al. (2009), environmental changes could eliminate or weaken a source of selection that is important for maintaining a specific trait in organisms.

The reduced salinity tolerance observed in KEN at 140 g/L suggests possible adaptive shifts or genetic drifts post-introduction, which may have led to a decreased salinity tolerance. Such evolutionary responses could be driven by the specific conditions of Kenyan saltworks, which differ from those of natural sources of SFB and GSL, thus selecting traits optimized for a narrow range of environmental salinities. The salinity range identified for optimal KEN performance has practical implications for *Artemia* production in Kenya. Culturing *A. franciscana* at salinities between 70 and 105 g/L could maximize the productivity and sustainability in Kenyan saltworks. This optimized salinity range aligns with findings from other regions, where specific salinity levels have been recommended to enhance *Artemia* culture performance (Castro-Mejía et al., 2011; Varó et al., 1993).

5 | CONCLUSION

The distinct response of KEN to high salinities requires further genetic and ecological studies to elucidate the mechanisms responsible for the differences. Genetic characterization could reveal alterations in KEN that account for reduced salinity tolerance, offering insights into the adaptive processes following its introduction. Investigating other ecological and physiological parameters would also deepen the understanding of how environmental factors and genetic make-up interact to shape population-specific responses to salinity.

AUTHOR CONTRIBUTIONS

Morine Ngarari: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; supervision; visualization; writing—original draft; writing—review and editing. **Sheban Hinzano**: Conceptualization; data curation; formal analysis; investigation; methodology; software; supervision; validation; visualization; writing—original draft; writing—review and editing. **Mary Opiyo**: Conceptualization; funding acquisition; methodology; project administration; resources; supervision; validation; visualization; writing—original draft; writing—review and editing. **Derrick Rugendo**:

Data curation; investigation; methodology; writing—review and editing. **David Midumbi**: Data curation; investigation; methodology; writing—review and editing. **Francis Okalo**: Conceptualization; funding acquisition; resources; writing—review and editing. **Betty Nyonje**: Conceptualization; project administration; supervision; writing—review and editing. **Charles Ngugi**: Supervision; writing—review and editing. **Charles Gatune**: Supervision; writing—review and editing.

ACKNOWLEDGEMENTS

The funding for this study was provided by Western Indian Ocean Marine Science Association (WIOMSA) through the Marine and Coastal Science for Management (MASMA) grant number MASMA/OP/2020/02. The Kadzuhoni Self-Help Group assisted with the salt ponds used for *Artemia* production. KMFRI provided laboratory equipment, technical teams and space for the success of this experiment.

CONFLICT OF INTEREST STATEMENT

The authors state that there are no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Morine M. Ngarari https://orcid.org/0000-0002-5611-1784
Sheban M. Hinzano https://orcid.org/0000-0003-3816-6930
Mary A. Opiyo https://orcid.org/0000-0001-9660-5888
Derrick G. Rugendo https://orcid.org/0000-0002-2569-9538
David O. Midumbi https://orcid.org/0000-0001-8584-8067
Francis A. Okalo https://orcid.org/0000-0002-0089-998X

PEER REVIEW

The peer review history for this article is available at: https://publons.com/publon/10.1002/aff2.166

ETHICS STATEMENT

The study was conducted following the standard operating procedures (SOPs) of the Kenya Marine and Fisheries Research Institute (KMFRI) guidelines for handling animals registered with the National Commission for Science, Technology and Innovation (NACOSTI) registration number NACOSTI/2016/05/001. The SOPs comply with the Prevention of Cruelty to Animals Act 1962, CAP 360 (Revised 2012) of the laws of Kenya and the EU regulation (EC Directive 86/609/EEC).

REFERENCES

Aalamifar, H., Agh, N., Malekzadeh, R. & Aalinezhad, M. (2014) A comparative study on the effect of different salinities on the survival, growth, life span and morphometric characteristics cyst of two parthenogenetic species of *Artemia* (gaav khooni wetlands of Isfahan, ponds around Lake Urmia) from Iran. *Asian Journal of Biological Sciences*, 7(6), 242–251. Available from: https://doi.org/10.3923/ajbs.2014.242.251

Agh, N., Van Stappen, G., Bossier, P., Sepehri, H., Lotfi, V., Razavi Rou, S.M. & Sorgeloos, P. (2008) Effects of salinity on survival, growth, reproductive and life span characteristics of *Artemia* populations from Urmia Lake and neighboring lagoons. *Pakistan Journal of Biological Sciences*, 11(2), 164–172. Available from: https://doi.org/10.3923/pjbs.2008.164.172

Amat, F., Hontoria, F., Ruiz, O., Green, A.J., Sánchez, M.I., Figuerola, J. & Hortas, F. (2005) The American brine shrimp as an exotic invasive species in the western Mediterranean. In: *Issues in Bioinvasion Science*: EEI 2003: a Contribution to the knowledge on invasive alien species, Singapore: Springer, pp.37–47. https://doi.org/10.1007/1-4020-3870-4_5

Bahr, A.S., Isroni, W. & Maulida, N. (2021) Hatching and harvesting techniques for *Artemia* cysts with different effects of salinity in the district of Situbondo, East Java. *IOP Conference Series: Earth and Environmental Science*, 718(1), 012037. Available from: https://doi.org/10.1088/1755-1315/718/1/012037

Castro-Mejía, J., Castro-Barrera, T., Hernández-Hernández, L.H., Arredondo-Figueroa, J.L., Castro-Mejía, 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. Available from: https://doi.org/10.15517/rbt.v59i1.3190

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(1–3), 175–188. Available from: https://doi.org/10.1023/B:HYDR.0000033124.49676.5c

Food and Agriculture Organization (FAO). (2022a) In: van Stappen, G. (Ed.), *Cultured Aquatic species information programme*. Rome: Fisheries and Aquaculture Division. Available from: https://www.fao.org/fishery/en/culturedspecies/artemia_spp/en

Food and Agriculture Organization (FAO). (2022b) *The state of world fisheries and aquaculture 2022*. Rome: FAO. Available from: https://doi.org/10.4060/cc0461en

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

Lahti, D.C., Johnson, N.A., Ajie, B.C., Otto, S.P., Hendry, A.P., Blumstein, D.T. et al. (2009) Relaxed selection in the wild. *Trends in Ecology and Evolution*, 24(9), 487–496. Available from: https://doi.org/10.1016/j.tree.2009.03.010

Lavens, P., Sorgeloos, P. & Food and Agriculture Organization of the United Nations. (1996) *Manual on the production and use of live food for aquaculture*. Rome: Food and Agriculture Organization of the United Nations.

Lavens, P., Tackaert, W. & Sorgeloos, P. (2007) International study on *Artemia*. XLI. Influence of culture conditions and specific diapause deactivation methods on the hatchability of *Artemia* cysts produced in a standard culture system. *Marine Ecology Progress Series*, 31. 197–203. Available from: https://doi.org/10.3354/meps031197

Mali, V., Sharma, B.K., Sharma, S.K. & Upadhyay, B. (2023) Analytical study of effect of salinity on growth and survival of *Artemia* nauplii in inland saline water of Didwana Lake, Rajasthan. *The Pharma Innovation Journal*, 12(3), 410–416. Available from: https://www.thepharmajournal.com

Manfra, L., Savorelli, F., Pisapia, M., Magaletti, E. & Cicero, A.M. (2012) Long-term lethal toxicity test with the crustacean *Artemia franciscana*. *Journal of Visualized Experiments*, 62, e3790. Available from: https://doi.org/10.3791/3790

Naegel, L. C. A. (1999) Controlled production of *Artemia* biomass using an inert commercial diet, compared with the microalgae *Chaetoceros*. *Aquacultural Engineering*, 21(1), 49–59. Available from: https://www.elsevier.nl/locate/aqua-online

Rodríguez-Almaraz, G.A., Zavala, C., Mendoza, R. & Maeda-Martínez, A.M. (2006) Ecological and biological notes on the brine shrimp *Artemia* (*Crustacea*: *Branchiopoda*: *Anostraca*) from Carmen Island, Baja California Sur, México. *Hydrobiologia*, 560(1), 417–423. Available from: https://doi.org/10.1007/s10750-005-1562-x

Triantaphyllidis, G.V., Poulopoulou, K., Abatzopoulos, T.J., Pérez, C.A.P. & Sorgeloos, P. (1995) International study on *Artemia* XLIX. Salinity effects on survival, maturity, growth, biometrics, reproductive and lifespan characteristics of a bisexual and a parthenogenetic population of *Artemia*. *Hydrobiologia*, 302(3), 215–227. Available from: https://doi.org/10.1007/BF00032111

Vanhaecke, P. & Sorgeloos, P. (1982) International study on *Artemia*. XVIII. The hatching rate of *Artemia* cysts —a comparative study. *Aquacultural Engineering*, 1(4), 263–273. Available from: https://doi.org/10.1016/0144-8609(82)90035-8

Vanhaecke, P., Tackaert, W. & Sorgeloos, P. (1987) The biogeography of Artemia: an updated review. *Artemia Research and Its Applications*, 1, 1–26.

Van Stappen, G. (2002) Zoogeography. In: *Artemia: basic and applied biology*. Dordrecht, the Netherlands: Kluwer Academic, pp. 171–224.

Varó, I., Taylor, A.C. & Amat, F. (1993) Comparative study of the effects of temperature, salinity and oxygen tension on the rates of oxygen consumption of nauplii of different strains of *Artemia*. *Marine Biology*, 117(4), 623–628. Available from: https://doi.org/10.1007/BF00349774

Veeramani, T., Santhanam, P., Manickam, N. & Rajthilak, C. (2018) Introduction to *Artemia* culture. In: *Basic and applied zooplankton biology*. Singapore: Springer, pp. 209–224. Available from: https://doi.org/10.1007/978-981-10-7953-5_7

How to cite this article: Ngarari, M.M., Hinzano, S.M., Opiyo, M.A., Rugendo, D.G., Midumbi, D.O., Okalo, F.A. et al. (2024) Salinity tolerance, growth and survival of three *Artemia franciscana* (Kellogg, 1906) populations under laboratory conditions. *Aquaculture, Fish and Fisheries*, 4, e166. https://doi.org/10.1002/aff2.166