Title: "First biogeographical survey of *Artemia* in Tanzania"

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Abstract

In an *Artemia* survey conducted along the coast of Tanzania between March and July 2017, 32 salt pans in Tanga, Dar es salaam, Pwani, Lindi, and Mtwara were assessed. Of all visited salt pans, 16 (50 %) had either *Artemia* biomass or cysts or both. Body length ranged from 4.0 mm to 9.5 mm, while the mean and modal lengths were 6.2 mm and 6.0 mm, respectively. Temperature ranged from 27.2 °C to 48.7 °C, salinity from 20 g L^{-1} to > 140 g L^{-1}, pH from 5.8 to 7.8, dissolved oxygen (DO) from 3.1 mg L^{-1} to 4.9 mg L^{-1}, water depth from 10 cm to 75 cm and conductivity from 42.0 ms cm^{-1} to 176.6 ms cm^{-1}. A bisexual population of *Artemia franciscana* is suggested. Observed cyst states included concave, biconcave, spherical and cracking cysts. This is the first to report on the occurrence of *Artemia* in Tanzania.

Key words: *Artemia*, biomass, cysts, individuals, salt pans

Introduction

Africa contributes 2.75% of the global salt production, with most of her techniques being conventional to primitive. Tanzania produces sea , inland lake and subsoil brine salts and the operations involve more than 6000 small and artisanal producers and less than a dozen medium-to-large producers (Assey et al., 2017; Mannar and Yusufali, 2013).

Finfish fingerling production in Sub-Saharan Africa especially African catfish (*Clarias gariepinus*) still faces underdevelopment due to the costs of formulated feeds and imported live foods such as *Artemia.* This results in low quantity and quality fingerlings for stocking grow out structures (Enyidi and Mgbenka, 2015). Inland aquaculture in Tanzania has shown an increase in the annual productions of tilapia species and African catfish (*Clarias gariepinus*) when combined, from 952 tonnes in 2010 to 3118 tonnes in 2015 (Rukanda, 2016). This fast growth opens opportunities for investing in hatchery production due to the increased demand of fingerlings (Mwaijande and Lugendo, 2015). Until 2015, 10 private and five public hatcheries were established for production of tilapia species, African catfish (*Clarias gariepinus*) and marine fish. The use of *Artemia* in some of these hatcheries has shown improvements in survival of African catfish (*Clarias gariepinus*) during the early life stages, which ensures availability of fry and fingerlings for stocking grow out ponds (Rukanda, 2016). Most of the currently operating hatcheries rely on imported *Artemia* cysts which are rather expensive. The cysts come partly from Kenya and mostly from Asia. Despite being expensive, the quality of the cysts is hardly guaranteed. Ornamental fish dealers in Tanzania are also using imported decapsulated *Artemia* cysts despite being uncertain of the quality. Many small-scale ornamental fish dealers cannot afford the costs of imported *Artemia*; therefore, they depend on the use of organically treated waters to grow their fish. Organic treatment of water such as using animal manures to grow zooplankton, does not ensure high nutritional quality and safety for the cultured fish, while the use of *Artemia* with either good or poor hatching guarantees higher production and safety (Lim et al., 2002).

To supply for the increased demand of *Artemia* due to increased aqua-hatchery operations, exploration for new sources has been a priority, an effort that has led to improved product quantity in the market. Despite this success, the demand of cysts has increased tremendously, and aqua-hatcheries are on the lead demanding higher quantities. Future estimates of *Artemia* rely on availability of information about their habitat ecologies (Lavens and Sorgeloos, 2000). To obtain information on habitat ecologies, it is of paramount importance to first survey and document biogeography of *Artemia.* Surveys and reviews on

Artemia distributions in different regions have depicted important alerts towards the future of *Artemia* production which must be ensured for aquaculture and salt production (Ben Naceur et al., 2009; Lavens and Sorgeloos, 2000; Mechaly et al., 2013; Vanhaecke et al., 1987). In Africa, records of *Artemia* exist for several regions including Algeria; Cape Verde; Egypt; Kenya; Libya; Madagascar; Mozambique; Morocco; Namibia; Niger; Senegal; South Africa and Tunisia. In these regions, *Artemia franciscana*; *Artemia salina*; and parthenogenetic *Artemia* populations have been reported (Ben Naceur et al., 2012; Kaiser et al., 2006). Most of these regions border the Mediterranean, this is not enough to cover the African distribution of *Artemia.* In East Africa, the occurrence of *Artemia* is mostly documented in Kenya, where it is reported that the currently available *Artemia franciscana* was introduced between 1984 and 1986 (Kaiser et al., 2006; Ogello et al., 2014a).

Due to the usefulness of *Artemia* in aquaculture and solar salt production, it is imperative to ensure its easy accessibility that will eventually reduce the cost of larviculture, result in exportation of processed *Artemia* in the future, in addition to ensuring good quality salt production. Although there is an increase in awareness of the importance of *Artemia* during the early life stages of fish in Tanzania, the need for research on its availability and application has hardly been attended and currently there is no information on its availability or distribution. This survey was therefore aimed at initiating records of *Artemia* in Tanzania through identifying *Artemia* sites by visiting salt pans along the coast of mainland Tanzania; determine the prospective size distribution; examine cyst hydration characteristics and observe the possible mode of reproduction. It is therefore a contribution to the biogeography of *Artemia* in Africa as it presents for the first time a record of *Artemia* availability in Tanzania.

Materials and Methods

Survey coverage

The survey coverage involved sampling between March and July, 2017 in Tanga, Pwani, Dar es salaam, Lindi and Mtwara regions along the coast of mainland Tanzania (Figure 1; Figure2). On an annual basis, there are no months without rain in the coast, but these regions experience varying rainfall patterns (Kabanda, 2018). A total of 32 salt pans were visited, and 15 of all visited salt pans were in Tanga. Ownership of the salt pans vary from company to family level. No further information on ownership of salt pans was collected.

Layout of the salt pans

The visited salt pans have the same layout described by Mani et al. (2012). Water firstly enters a reservoir pond during high tide. After attaining desired salinity, the water is released into evaporation ponds. The resulting brine is released into crystallization ponds in which salt crystallizes out and then it is harvested.

Collection of Artemia and environmental parameters

On arrival at each salt pan, all ponds were surveyed for presence of *Artemia* biomass and cysts, then samplings were done in ponds where *Artemia* biomass and/or cysts were spotted. *Artemia* biomass was collected by using a plastic tea strainer while cysts were collected by passing a piece of cloth slightly below the water surface (Figure 3). Sampling was done in only one spot in a pond with *Artemia* biomass and/or cysts. For salt pans with more than one pond containing *Artemia*, the sampling was done in only one pond, and in more than one pond where *Artemia* biomass and cysts were found in different ponds. The samples were fixed in 98% ethanol in eppendorf tubes and stored on ice for transport. For all visited salt pans, geographical coordinates acquired by using a handheld navigator (GARMIN GPSMAP 62s) were recorded. Water depths were measured by using a measuring tape, while temperature, conductivity and pH were measured by using a water multiparameter meter (HANNA HI 98194). Salinity was measured by using a hand refractometer with a maximum of 140 g L^{-1}, in cases where salinity exceeded this range, the readings were recorded as > 140 g L^{-1}. The measurements were done at randomly different times of the day and different depths depending on the pond(s) in which *Artemia* biomass and/or cysts were found. Neither measurements of salt pan and pond sizes nor observations of the number of ponds in the salt production line were conducted.

Microscopic observation of cysts

To establish a first step in understanding the relationship between external salinity, metabolic activity and physical state of cellular water in cysts as previously described (Drinkwater and Crowe, 1991), the shape (concave, biconcave or spherical) and cracking of cysts were examined by using a portable field microscope (Bressor, Biolux, German).

Size distribution, descriptive statistics and map creation

Individual total body length was measured using a ruler for a sample of 127 individuals which represented all visited locations. Descriptive statistics were conducted in the statistical computing environment of R version 3.4.0. Map was created using ArcGIS® software (Environmental Systems Research Institute (ESRI)).

Results

Geographical distribution

In all regions with exception of Mtwara, *Artemia* biomass and cysts were found (Figure 4), where 16 (50 %) of all salt pans visited had either both *Artemia* biomass and *Artemia* cysts or only one stage (Table 1). *Artemia* biomass was found mainly in the evaporation ponds while *Artemia* cysts were found as floating brownish mats mainly in the corners of crystallization ponds.

Environmental parameters

During the survey, both sunny and rainy days were experienced. There were less rainy days during the survey in Tanga and more rains were experienced during visits in Pwani, Dar es salaam, Lindi and Mtwara. Recorded temperatures in the salt pans ranged from 27.2 °C to 48.7 °C, salinity ranged from 20 g L^{-1} to > 140 g L^{-1}, pH from 5.8 to 7.8, dissolved oxygen (DO) from 3.1 mg L^{-1} to 4.9 mg L^{-1}, depth from 10 cm to 75 cm and conductivity from 42.0 ms cm^{-1} to 176.6 ms cm^{-1} (Table 2). Cysts were found in shallow ponds though in some ponds, both *Artemia* biomass and cysts were found, these ponds were also shallower than those with only *Artemia* biomass. Salinity did not seem to separate *Artemia* from their cysts since *Artemia* biomass could also be found both in the highest and lowest salinities recorded. No *Artemia* biomass and cysts were found at temperatures below 35 °C.

Size distribution

Individual lengths were randomly distributed through all the visited regions. The modal class was 6.0 to 6.5 mm and average length was 6.2 mm, while minimum and maximum lengths were 4.0 mm and 9.5 mm, respectively (Figure 5).

Sexuality

Males with claspers for securely holding females during mating, females carrying ovisacs with oocytes and clasped swimming couples were observed. These are important indicators to identify sexuality in *Artemia* (Figure 6).

Microscopic observation of cysts

Under the microscope, cysts had either concave, biconcave or spherical shapes while some cysts found together with spherical cysts had cracks (Figure 7).

Discussion

Morphometrics of cysts, nauplii and adult have been used to characterize *Artemia* and discriminate developmental stages and sexes (Ben Naceur et al., 2010; John et al., 2004).The size distribution reported in this study has most individuals less than the length *Artemia salina* which ranged from 8.0 mm to 12.0 mm (Dumitrascu, 2011). A higher mean size of 10.6 ± 1.06 mm was reported for a parthenogenetic *Artemia* population in India (John et al., 2004). Despite the reported significant effects of different salinity levels on total body length of *Artemia franciscana* (Mueller et al., 2016), the time span, amount of information available and organization of this survey may not provide sufficient information to suspect salinity as the cause of the observed distribution in total body length.

Two observations in this survey suggest presence of a bisexual population of *Artemia* in Tanzania: 1.) morphology which includes ovisacs with eggs as seen in females and claspers in males, 2.) mating behavior which in which a male clasp on a female. These explanations agree with previous descriptions of sexuality in an *Artemia* population, in which females are recognized by their ovisacs, males by their claspers. The mating behavior where a male clasps on a female has also been documented (Lent, 1977; Sugumar, 2010). However, the current speculation that the population is bisexual is not sufficiently supported since it is also possible for both types of reproduction to occur in coexisting species, that is; presence of males and females in sexual reproduction, but at the same time parthenogenetic stocks in which females and rare non-functional males are present (Ben Naceur et al., 2012; MacDonald and Browne, 1989; Mechaly et al., 2013).

Ovoviviparity occurs when environmental conditions are favorable whilst survival in temporarily unfavorable conditions is assured through oviparity in which the *Artemia* produce resistant cryptobiotic eggs commonly known as cysts (Mechaly et al., 2013). This argument may support the presence of cysts in some of the visited salt pans especially one in which the salinity was above 140 g L^{-1}. Encountering cysts in salinities as low as 26 g L^{-1} was not considered unusual as raining had just started, and it was in these salinities where the spherical and cracking cysts were found. Spherical cysts indicate that hydration was taking place while the cracking cysts meant hatching was taking place and they were in the breaking stage as explained by Van Stappen (1996).

In addition to the natural distribution, *Artemia* are also distributed through purposeful introductions by man for improving salt production but also for production of live food to be used in aquaculture (Van Stappen, 1996). Purposeful introduction of *Artemia* in solar salt operations is exemplified by Kenya in East Africa (Ogello et al., 2014b). Following the genetic closeness revealed in their study, Ogello et al. (2014b) suggested that the *Artemia* in Tanga comes from Kenya through introductions by Kensalt management. Kenya and Tanzania share a coastline; therefore, it is wise to speculate that the bisexual *Artemia* from Tanzania is *Artemia franciscana*. However, such speculations need to be confirmed by an extensive *Artemia* characterization study in Tanzania.

Apart from salt pans, *Artemia* can be found in inland salt lakes and coastal lagoons where salinity levels are above 40 g L^{-1} (Kaiser et al., 2006; Kappas et al., 2004; Vanhaecke and Sorgeloos, 1980). Such water bodies are also found in Tanzania and they include inland saline lakes such as lakes; Natron, Manyara, Eyasi, Kitangiri, Balangida, Singidani, Balangidalelu, Basotu and Basoda. There are however knowledge gaps on chemistry and aquatic science in these lakes (Nzaro, 2000; Philip and Mosha, 2012). It is thus worthy to speculate the possibility that *Artemia* distribution in Tanzania is beyond the coastal salt pans.

The importance of *Artemia* in solar salt works is also acknowledged by owners of the surveyed salt pans. Despite being unaware of the mechanisms behind, the owners and workers acknowledged harvesting good quality salt in times with high densities of *Artemia* in the crystallization ponds. Some of the owners reported to routinely monitor *Artemia* and whenever they disappear, workers visit neighboring salt pans to collect *Artemia* biomass to inoculate in their own salt pans. The though simple understanding of the role of *Artemia* in the production of good quality salt agrees with Tackaert and Sorgeloos (1993) who reported on control of algal blooms by *Artemia* through feeding and provision of suitable substrates for halophilic bacteria which play a role in lowering the viscosity of water. Successful optimization of the interaction can thus result in improved salt production but requires proper biological management of the salt pans for good quality salt (Tackaert and Sorgeloos, 1993).

Conclusion and recommendations

This is the first record on the availability and distribution of *Artemia* in Tanzania. It is believed that, the initiative will serve as an alert for future explorations for sustainable production of *Artemia* in Tanzania. Therefore, the authors recommend: further in depth investigations to unravel more about the distribution with regards to time, space and geographical positions of sites; assessing the reproductive mode with larger spatial and seasonal diversities using better techniques; studying the species composition with the help of molecular techniques and spatially diverse studies; social studies on the awareness and scientific studies on the integration of local *Artemia* in salt production and its aquaculture application in Tanzania.

Acknowledgements

This study was financially supported by DANIDA Fellowship Center under its Building Stronger Universities (BSU) phase 2 programme. Local governments which provided permission and fisheries officers who escorted the survey in Tanga, Pwani, Dar es salaam, Lindi and Mtwara deserve gratitude from the accomplishment of this work.

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Figure 1. Map of Tanzania showing the surveyed regions (circled in red). (One Planet Nations Online 2017).

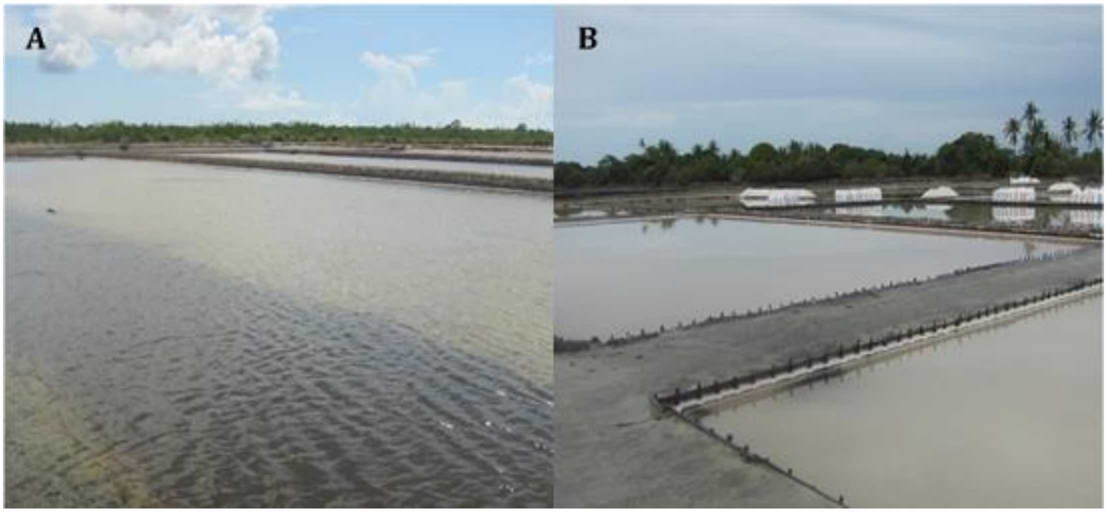

Figure 2. Evaporation ponds (a) and crystallization ponds (b) of a saltpan in Tanga region.

Figure 3. *Artemia* individuals captured in a plastic tea strainer (a) and *Artemia* cysts floating in a brownish mat at the corner of a crystallization pond (b).

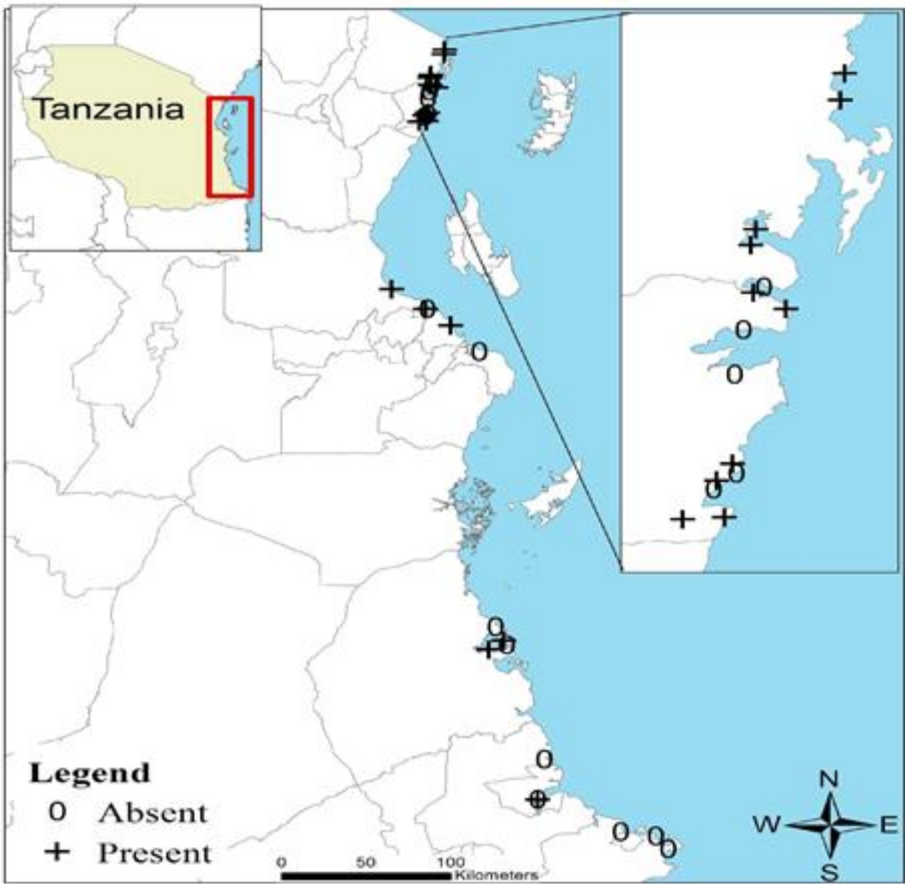

Figure 4. Geographical locations of all visited sites along the coast of mainland Tanzania. The + and 0 signs indicate the presence and absence of *Artemia*. A site with either *Artemia* biomass only or Artemia cysts only is awarded +, similar to a site with both *Artemia* biomass and cysts.

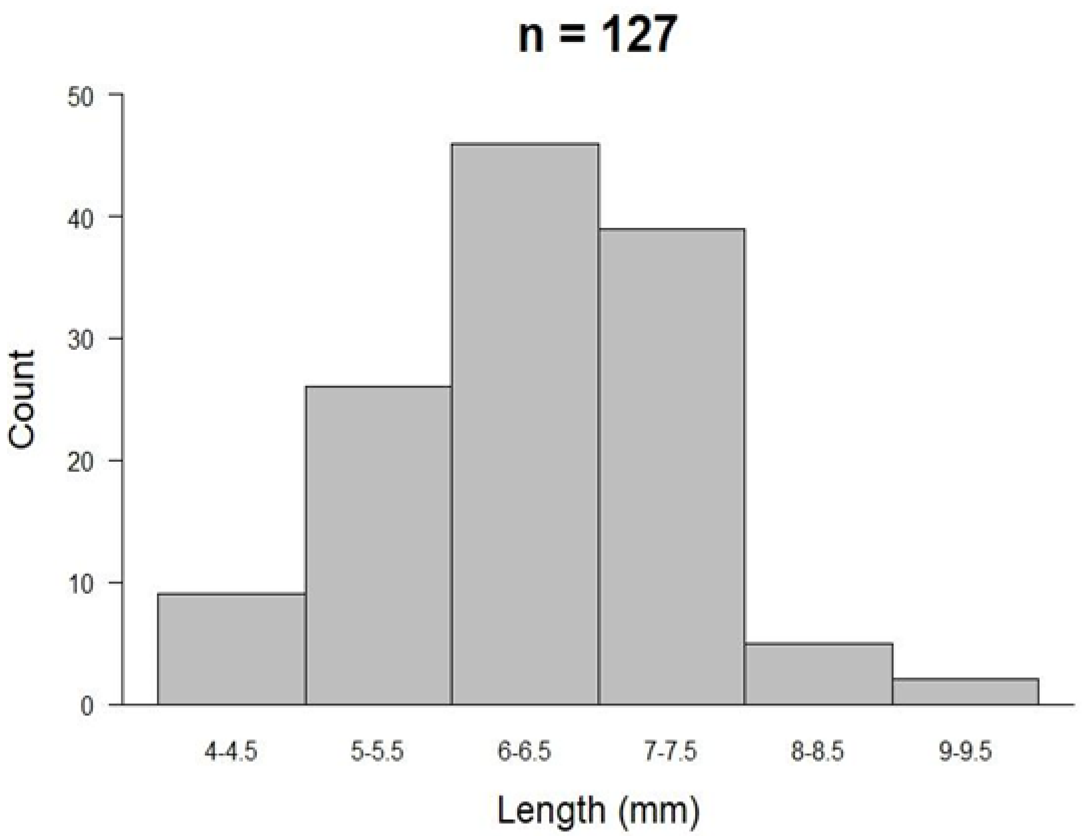

Figure 5. Frequency distribution of the total lengths of 127 *Artemia* individuals from random samples of all biotopes with *Artemia*.

Figure 6. *Artemia* females, males and a mating couple as found in one of the salt pans.

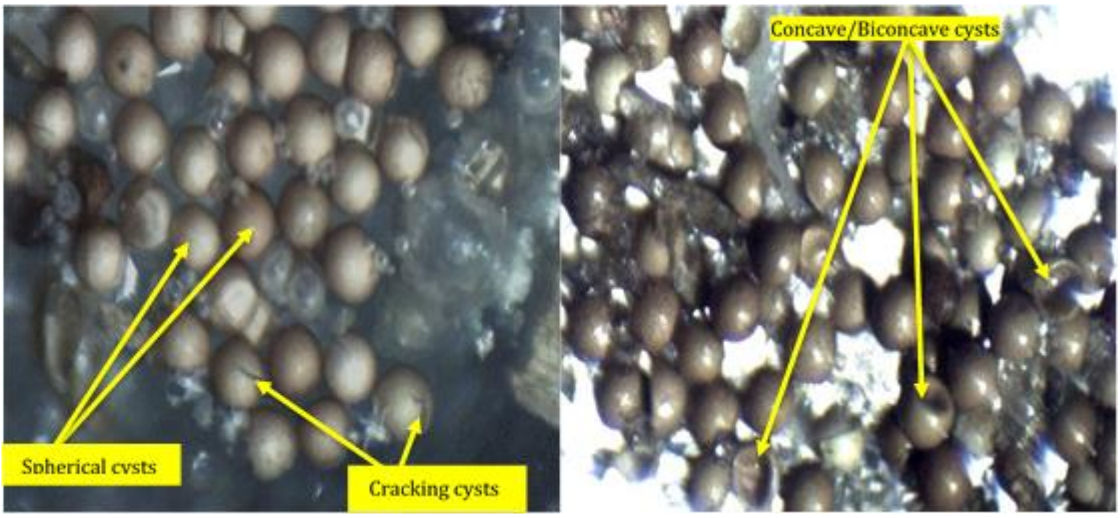

Figure 7. Microscopic view of *Artemia* cysts collected during the survey.

Table 1. *Artemia* stages in the visited salt pans

***Artemia* stage**	**Number of salt pans**	**Percent (%)**
Biomass only	13	81
Cysts only	1	6
Biomass and cysts	2	13
Total	16	100

Table 2. Visited biotopes along the coast of mainland Tanzania, with their characteristics observed during the survey

Geography of biotopes						*Artemia*			Physio-Chemical Properties of Water					
Region	S/No	Site	X (UTM)	Y (UTM)	ASL	M	F	Cysts	Temp (°C)	Salinity (g L^{-1})	Cond. (ms cm^{-1})	pH	DO (mg L^{-1})	Depth (cm)
Tanga	1	Mwarongo	508851	9421695	15.02	+	+	-	38.5	85	132.5	7.2	3.1	75
	2	Geza Ndani	504915	9421431	15.16	+	+	-	47.6	> 140	135.4	7.2	4.1	44
	3	Saadani Tongoni	507816	9425223	15.17	-	-	-	42.2	130	146.6	7.2	3.7	10
	4	Maere	508082	9426295	15.57	+	+	-	37.9	135	159.9	7.35	3.9	20
	5	Kivindani	509945	9427214	12.00	-	-	-	36.6	130	156.0	6.8	3.2	20
	6	Machui	509576	9428391	16.22	+	+	-	36.7	85	174.6	5.8	2.9	20
	7	Mwacheji	510450	9445172	15.81	-	-	-	34.5	20	133.5	6.9	4.0	30
	8	Chongoleani	514416	9447802	15.57	-	-	+	35.0	26	42.0	7.4	4.4	10
	9	Mpirani	511338	9449805	15.53	+	+	+	40.1	40	102.5	7.1	4.6	20
	10	Moa-Mayomboni	519702	9477295	16.01	+	+	-	38.2	110	105.9	7.5	4.9	60
	11	Moa	519322	9473946	16.30	+	+	-	39.5	95	129.2	6.5	4.5	10
	12	Kibiboni	511050	9455735	17.44	+	+	-	36.8	100	127.2	6.6	4.0	20
	13	Doda	511508	9457718	15.19	+	+	-	37.2	95	112.0	6.8	4.1	30
	14	Kizingani	512337	9450529	15.68	-	-	-	37.9	60	89.9	7.56	4.6	20
Pwani	15	Kingani	509651	9439580	16.51	-	-	-						
	16	Nunge	488307	9290691	16.15	+	+	-	36.1	140	118.3	7.3	3.6	15
	17	Changwahela	508386	9275290	15.80	+	+	-	37.7	140	130.4	7.0	4.3	30
Dar es Salaam	18	Ununio	509386	9275290	15.20	-	-	-	48.7	40	98.9	7.1	3.2	30
	19	Kunduchi	523173	9262328	15.27	+	+	-	45.2	> 140	176.6	6.0	3.0	10
	20	Kigamboni	540056	9241921	15.69	-	-	-	39.9	65	65.2	7.8	3.5	30
Lindi	21	Msakara	549358	9027257	17	-	-	-	34.3	140	78.3	6.9	3.8	30
	22	Lundu	545439	9009400	5	+	+	-	41.4	130	183.2	6.1	3.8	30
	23	Mshindo	554118	9016373	1	+	+	-	39.4	140	186.2	6.2	4.0	30

	24	Mjinini	556071	9013137	4	-	-	-						
	25	Machole 1	573752	8892347	18	-	-	-						
	26	Machole 2	573751	8892190	1	+	+	+	36.7	> 140	171.1	6.6	4.0	15
	27	Mchinga	577763	8923529	22	-	-	-						
Mtwara	28	Ufukoni	577703	8923526	-31	-	-	-						
	30	Mitengo	642703	8863533	2	-	-	-						
	31	Cheleweni	622226	8867057	-4	-	-	-	27.2	53.6	77.2	7.5	4.7	27
	32	Lihanje	649972	8853568	-6	-	-	-	30	98	112.2	7.6	6.6	30

Key: X (Longitude); Y (Latitude); UTM (Universal Transverse Mercator); ASL (Altitude Above Sea Level); M (Male); F (Female); Temp (Temperature); Cond. (Conductivity); DO (Dissolved oxygen)