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Natural Production of *Artemia* in the Evaporation Ponds of a Water Treatment Plant in Saudi Arabia

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Abstract

Live food such as *Artemia* is considered to be an essential part of many crustacean and finfish hatcheries. The natural occurrence of the brine shrimp *Artemia* is reported for the first time in highly saline evaporation ponds of the water treatment plant at Al-Qassim Research Station of King Abdulaziz City for Science and Technology (KACST) in Saudi Arabia. The evaporation pond produced, during a 3-month period, more than 37 kg of *Artemia* cysts /3600 m^2 (wet weight). The highest biomass production (over 19 and 7 kg of wet and dry cysts/3600 m^2, respectively) was collected on March. The hatching percentage decreased gradually throughout the trials and ranged from 73.4%-87.3%. The water quality of the ponds is also discussed in relation to the occurrence of *Artemia*.

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Introduction

A major task for aquaculturists is the choice of suitable foods and the development of feeding regimes for larvae in devising a protocol for the culturing of fish species (Tamaru et al. 1999). Live foods are among the most convenient and often essential food sources for the larvae of some cultured species. Live foods like the brine shrimp *Artemia* and rotifers are still recognized as the best foods for fish and crustaceans (Hoff and Snell 1987). Two major concerns among aquaculturists are: an appropriate size of the live food organism for first feeding of larvae and a regular supply of large numbers of live feed. Two live feeds, the rotifer *Brachionus* sp. and the brine shrimp *Artemia* satisfy both the size and numerical requirements (Lubzens et al. 1989). Newly hatched *Artemia* have been widely used for many years both by commercial and amateur fish breeders. This is due to their high nutritional value and their availability off the shelf when most needed. However, the nutritional quality of live planktonic feeds used in aquaculture is an important factor for the survival and optimum growth of larvae and juveniles of fishes, crustaceans, and mollusks (Szyper 1989). Though prepared diets have been developed and are available in the market for the larval culture of many fish species, they are not completely effective, and therefore live foods are still used (Webster and Lovell 1990).

Artemia is an important feed in the aquaculture industry (Landau and Sanchez 1991). It is reported that *Artemia* nauplii comprise an estimated 85% of all live foods fed to fish larvae worldwide (Sorgeloos et al. 1993). The brine shrimps of the genus *Artemia* are typically found in saline/hypersaline environments (Triantaphyllidis et al. 1998; Van Stappen 2002) and are distributed all over the world. They have been recorded in over 600 coastal and inland sites world wide (Triantaphyllidis et al. 1998; Van Stappen 2002). *Artemia* cysts are dispersed by seagulls, ducks and flamingos (Hoff and Snell 1987). In addition to its ecological interest, the brine shrimp is also used as food additive for domestic livestock. It is consumed by humans in Africa and Thailand (Hoff and Snell 1987) and is needed in solar saltworks to produce high quality salt (Davis 1980; Landau and Sanchez 1991). It has been used as a live food for fish culture since the 1920's (Hoff and Snell 1987). Commercially, *Artemia* cysts are being preserved in dry form, placed in vacuum-packed containers and sold worldwide by different enterprises in North and South America, Europe, Australia and Asia. Since the aquaculture industry is steadily growing and contributing significantly to the world fish production (in addition to the contribution by the fish capture sector), commercial exploitation of *Artemia* has become a big business. Several sources of *Artemia* can be found in different geographical regions such as for example those in San Francisco Bay and Great Salt lake (USA), Macau (Brazil), Manaure and Galera Zamba (Columbia), Chaplin Lake (Canada), Buenos Aires (Argentina), Larnaca (Cyprus), Barbanera (Spain), Shark Bay (Australia), Tuticorin (India), Tianjin, (China), and Margherita di Savoia, (Italy), (Hoff and Snell 1987). So far, no reports are available on the natural occurrence of *Artemia* in Saudi Arabia. This paper describes the natural production of *Artemia* in the evaporation ponds of a water treatment plant.

Materials and Methods

Occurrence of Artemia at Al-Qassim Research Station

There are no reported instances of natural occurrence of *Artemia* in the saline environments of Saudi Arabia. For the first time since the establishment of Fish Culture Project at Al-Qassim Research Station of King Abdulaziz City for Science and Technology (KACST) in 1992, the station has produced *Artemia* cysts as well as biomass from the evaporation ponds of the water treatment plant of KACST (using reverse osmosis method). These evaporation ponds of the water treatment division of KACST serve as the collecting area for the wastewater of the treatment plant. This wastewater, discharged from the water treatment plant, has high salinity (>100 g/L). Occasionally, additional wastewater from the fish culture project is used to increase the water volume of the evaporation pond to satisfy the *Artemia* production (both cysts and biomass). The salinity of these evaporation ponds satisfies the requirement of *Artemia* for propagation and production of cysts in adequate quantities for the Al-Qassim Stations' requirements. The presence of *Artemia* in the evaporation ponds was traced from the daily draining of used water from the fish culture. The wastewater from the fish culture is drained to a series of sedimentation tanks where the bigger colloidal particles of the water are allowed to settle before moving to the irrigation tank. The irrigation tank is the main source of water for the plants surrounding the fish culture project and the excess water from the irrigation tank eventually overflows to the evaporation pond of the water treatment division.

The used water from the greenhouse carries later stage *Artemia* nauplii, which are too big for the fry or larvae to swallow. These *Artemia* instars are carried to the sedimentation tank and then to the irrigation tank and finally to the evaporation ponds of the water treatment division of the Research Station.

Usually, fish-breeding activities in Al-Qassim Research Station take place in summer, and *Artemia* is one of the feeds being given to the fish larvae. However, the fish larvae cannot utilize all *Artemia* nauplii in the larval rearing tanks. During the daily water management, some of the unconsumed *Artemia* nauplii go with the water to the irrigation tank and evaporation pond as well.

Experimental pond conditions

This study was conducted in three artificial ponds at a fish culture station located in the Al-Qassim region of Saudi Arabia. These ponds were constructed in 1995 and are completely dependent upon the supply of Reverse Osmosis Plant wastewater for water sources throughout the year. The layer of bottom mud is between 6 and 8 cm. Water was added in the pond to compensate for the loss from evaporation and seepage. The surface of each pond is 3600 m^2 with an average depth of 1.3 m. The water level ranges between 80 and 100 cm.

Physicochemical characteristics

The water quality in the evaporation ponds was determined on a weekly basis following APHA (1998). Surface water temperatures, dissolved oxygen (DO), pH and total dissolved solids (TDS) of the ponds were measured using a Universal Pocket Meter Multiline P4 **(**WTW, Weilheim, Germany). Salinity was determined using a refractometer (A366ATC, Japan). While NO_2-N and hardness were recorded using a HACH DR/2000 analysis unit (HACH Co., Ames, USA). All measurements were taken between 0800 and 0900.

Collection, cleaning and drying of the harvested cysts

Artemia cysts were collected periodically from the water surface of all the evaporation ponds. Plankton net (mesh size 100 µm, aperture 45x 26cm; length,100cm) was used for scooping the *Artemia* cysts, which float along with the detritus in the pond. The collected cysts were placed in buckets and then transported to the laboratory for cleaning and drying.

The newly harvested cysts generally mix with detritus and other impurities that float on the water surface. Cleaning and separation of cysts from detritus and other forms of impurities are the first steps carried out before drying the cysts. The following steps were taken in cleaning and drying the cysts.

The newly harvested cysts (along with the detritus) were screened through 1000 to 75 µm mesh size stain less steel screen and resting on a large empty basin. Flowing freshwater was used to flush the cysts and to eliminate the debris. Based on the ocular inspection, the presence of cysts was no longer visible in the sieve, the detritus was discarded. After the first stage of separating the cysts from bigger detritus, the cysts were collected again using plankton net (mesh size 100 µm). The same process of allowing flowing freshwater on the batches was repeated to drain the cysts to the collecting basin, leaving behind the detritus. Afterwards, the cleaned cysts were placed in brine and were left to soak overnight to dehydrate and to allow the precipitation of smaller particles such as sand and other impurities. The following day, the cysts were carefully drained using plankton net (mesh size 100 µm) and the wet weight of the cysts was recorded. Extra care was exercised while draining the brine to avoid disturbance of the precipitants, which contain most of the smaller particles of impurities. The cysts were then spread thinly on a cloth resting above a screen to allow excess water to leach. The cysts were air- dried inside the greenhouse. The dried cysts were weighed, recorded and stored in a cool and dry place (20-22°C) for future use.

Storage and hatching percentage

Immediately after the *Artemia* cysts were dried, 45 bags of cysts (5g each) were packaged in sterile polyethylene bags and stored in walk-in freezer maintained at -20±1ºC. Samples of the frozen *Artemia* cysts were examined for hatching percentage every week for the first month and then every month for a period of 12 months. At each time, three bags of frozen cysts were randomly taken from the freezer and evaluated for hatching percentage. *Artemia* cysts were at 27-28 ºC under continuous illumination and aeration, according to Sorgeloos et al. (1986). Percentage hatch was calculated after 48h.

Results and Discussion

This is the first report on the production of *Artemia* in the evaporation ponds of a water treatment plant in Saudi Arabia. The mean values of water temperature and other water quality parameters of the evaporation ponds for one complete year are given in table 1.

Table 1. Ranges and mean values of water quality parameters in the evaporation ponds

Parameters	Range	Mean ± SD
Temperature (°C)	11.2-31.9	25.43±6.72
Dissolved Oxygen (mg/L)	5.1-10.1	8.19±1.39
pH	6.4-8.9	7.72±0.73
Salinity (ppt)	76.0-125.0	109.88±13.55
Turbidity (NTU)	2.30-12.4	4.47±3.35
Total Dissolved Solids (mg/L)	14310-65360	36115.83±16205.48
Alkalinity (mg/L as $CaCO_3$)	102-193	186.92±20.77
Total hardness (mg/L as $CaCO_3$)	9020-15371	11773.58±2073.96
Sodium (mg/L)	25000-45100	35962.5±8577.1
Potassium (mg/L)	568-870	746±146.4
Calcium (mg/L)	816-1440	1189.5±282.79
Magnesium (mg/L)	2181-2952	2591±346.78
Ammonia (mg/L)	0.05-0.85	0.51±0.38
Nitrite (mg/L)	0.43-0.82	0.57±0.12
Sulphate (mg/L)	7832-11520	9963±1752.5
Chloride (mg/L)	46179-75967	64486.8±13998.9
Phosphates (soluble) (mg/L)	0.05-0.15	0.1±0.05
Silica (mg/L)	2.5-4.5	3.5±1.15

The water temperature in the *Artemia* culture pond ranged between 11.2 and 31.9°C. For several *Artemia* populations, the optimum temperature range is between 19 and 25°C (Dhont and Lavens 1996). The pH of the water was overall alkaline (average 7.72). Dhont and Lavens (1996) pointed out that the tolerance of *Artemia* ranges between 6.5 and 8.0. The levels for dissolved oxygen (DO) on the average were 8.19 mg/L. *Artemia* biomass production is inhibited only if the concentration of oxygen is lower than 2 g l^{1} (Dhont and Lavens 1996). The total dissolved solids (TDS) ranged between 14310 and 65360 with an average of 36115.83±16205.48. The mean value of the water salinity was 109.88 g/L. Sorgeloos et al. (2001) have reported that the optimal salinity for *Artemia* production is in the range of 100-150 ppt. However *Artemia* tolerates even higher salinities as brine shrimps have been reported at salinities as high as 340 g/L (Abatzopoulos, et al. 2006). Moreover, *Artemia* is an excellent osmoregulator maintaining the salt concentration of its body tissues within a narrow limit of 9 ppt (Hoff and Snell 1987). Therefore, fluctuating salinity does not seem to be a problem. Besides temperature and salinity, other water quality parameters also govern the survival of *Artemia*. Studies indicate that culture methods for *Artemia* are varied and include the rearing of these organisms in environments where high levels of ammonia may be encountered, such as wastewater facilities (McShan et al. 1974). The average ammonia concentration was lower than 0.5 mg/L in the water of the evaporation pond, although diurnal fluctuation cannot be ruled out. *Artemia*, appears to be more tolerant to ammonia than most other aquatic organisms (Landau and Sanchez 1991). Dhont and Lavens (1996) pointed out that *Artemia* are tolerant to much higher concentrations of nitrite and ammonia than those recorded in the present work.

Overall, the water quality parameters of the evaporation ponds were within the optimum range required by *Artemia* (Hoff and Snell 1987; Dhont and Lavens 1996; Sorgeloos et al. 2001). Triantaphyllidis et al. (1998) have reported that *Artemia* are found in a wide variety of harsh environments world wide.

The production of *Artemia* cysts from the evaporation ponds for the period from March to May 2002 is presented in table 2. The evaporation ponds produced, during a 3 month periods, more than 37 kg (wet weight) of *Artemia* cysts /3600 m^2. The highest biomass production (over 19 and 7 kg of wet and dry cysts/3600 m^2 respectively) was collected in March, after which the collection declined. Cyst yields, during the limited period of the experiment, demonstrated a relatively high production potential (Baert et al. 1997; Zmora et al. 2002). The decline in cyst production could be attributed to the mortality of *Artemia* in the ponds. As described earlier, *Artemia* are drawn towards strong light, which causes increased swimming activity and greater energy expenditure, resulting in slower growth rates. In low light, *Artemia* spread out in the water column swimming slowly and achieving more efficient food conversion (Hoff and Snell 1987). In the case of our experiments, heavy mortality was observed and thereby no eggs could be harvested. Table 2 shows wet and dry weights and moisture loss of the *Artemia* cysts collected at different dates in the evaporation ponds.

Table 2. Cyst collection data from the evaporation ponds

Date of collection	**Quantity**		**% Moisture loss**
	Wet weight (grams)	Dry weight (grams)	
13 March 2002	8210.30	3100.15	62.24
27 March 2002	11448.27	4236.47	62.99
10 April 2002	6842.86	2552.18	62.70
24 April 2002	5638.71	2087.25	62.98
8 May 2002	2967.48	1093.72	63.14
22 May 2002	2174.90	800.44	63.19
Total	37282.52	13870.21	377.24
Average	6213.75	2311.7	62.87

The difference between the wet weight and the dry weight ranges from 62.24 to 63.19%. The total quantity of cysts collected from March to May 2002 was 13.870 kilograms (dry weight). From table 2 it is evident that as summer time approached lesser amounts of cysts were collected.

Table 3. Hatching percentage of the locally produced *Artemia* cysts at varying frozen durations

Duration	Hatching percentage
Week 1	87.3
Week 2	85.7
Week 3	85.4
Week 4	86.0
Month 2	85.2
Month 3	80.8
Month 4	83.1
Month 5	80.2
Month 6	79.3
Month 7	80.1
Month 8	77.8
Month 9	75.9
Month 10	76.0
Month 11	75.6
Month 12	73.4

Hatching percentage of the cysts is shown in table 3, which decreased gradually throughout the trials and ranged from 73.4%-87.3%. The duration of the freezing also affects the hatching percentage of the brine shrimps. Therefore, the hatching percentage was tested after different freezing durations. Successful hatching remained around 80% up to the sixth month freezing and declined slightly till the 12th month. The hatching percentage never dropped to below 70%. Usually, supply of *Artemia* cysts from other countries entails long negotiations and higher cost of purchase as well as transport. This finding will be significant to both marine and freshwater aquaculture as it will pave the way to boost fry production of both fish and shrimp species presently cultured in Saudi Arabia, and will eventually increase aquaculture production. The dependency of the fish farmers in Saudi Arabia to imported *Artemia* cysts will be reduced thereby increasing their margin of profit. Furthermore, these findings can be another investment opportunity for aquaculture entrepreneurs in the future. This study aims to provide the information that *Artemia* can be cultured successfully in Saudi Arabia considering the enormous hyper saline areas available both inland and along the coasts of the Red Sea and the Arabian Gulf.

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