

INTEGRATION OF *ARTEMIA* IN INDIAN SALT WORKS- ECONOMIC OPPORTUNITIES

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EXTENDED ABSTRACT

Artemia, which thrives in hyper saline waters, can very well be integrated in solar salt works, offers excellent scope for getting nutritional live feed and world quality salt production. In many salt works, hydro-biological activities and their management are essential for the production of high quality and quantity salt. One of these is the algal blooms, which increases solar heat absorption and aids rapid evaporation. This leads faster production of higher quantities of salt crystals. However, a kink arises when the algal blooms metabolization was not in time. Otherwise algal excretions and decomposition prevent early precipitation of gypsum, contaminate the salt, reduces its quality and leads to precipitation of smaller salt crystals. The presence of *Artemia* controls the algal blooms, preventing the contamination. In addition *Artemia* metabolites provide nutrients for the development of microbe called halobacterium, red bacteria, within the crystallization ponds, increase heat absorption thereby increase the quantity of salt production. Proper management of *Artemia* population in solar salt works lead to optimal production of salt quality, quantity and lead to the exploitation of valuable by-products, including cysts and biomass. Further the nutritional value of *Artemia*, nauplii seem to be determined by their content of essential fatty acids

In our field study it was very successful in introducing Vietnam *Artemia* strain in Indian salt pond and produce higher amount of *Artemia* biomass and cysts. In this study we report size, hatching rate and efficiency of harvested cysts after 5 months production in inoculated salt pans were high quality and quantity and found to be great commercial importance. The fatty acid profiles of *Artemia* cysts showed high level of eicosapentaenoic acid 20:5 n-3 essential fatty acids in all produced cysts batches and related to adequate feed condition existed in the natural habitat. This study gains an importance in the cultivation of halo tolerant micro algae *Dunaliella salina*. Under stress conditions such as high salinity, light intensity and high temperature allowed to accumulate high beta carotene which reflected in the nutritive value of *Artemia*. As the resource are available in Indian salt pans, it is imperative to use the resource on commercial grounds.

KEYWORDS: salt works, *Artemia*, *Dunaliella salina*, biological management, fatty acid profile, nutritive value.

1. INTRODUCTION

The role of biological management in optimizing the quality and quantity of solar produced salt was examined by many workers. (Davis, 2006, 2000, Magana et.al., 2005, Tackaert and Soregeloos, 1992). Solar salt works are man-made artificial ecosystems

that are highly vulnerable to algal blooms that can contaminate the salt with gypsum and other insoluble materials and lead to reduced evaporation. The introduction of *Artemia*, into these systems in sufficient numbers can control these algal blooms which should lead to improved salt production and also provide opportunities for the harvesting of cysts and biomass (Ahmed S.U, et al, 2000). *Artemia* are known to exist throughout the world in saline or brackish waterways. Typically these waters have salinities within the ranges of 70-250 ppt and water temperatures 5-35 °C. *Artemia* thrive in the wild where conditions are such that there are virtually no predators and there is limited competition for food. The ability to live in these hostile conditions can be attributed to the physiological adaptations that they developed due its efficient osmo-regulatory system, to cope with low dissolved oxygen levels at high salinities and to produce 'dormant cysts' to counter adverse environmental conditions.

Artemia are used throughout the world in prawn and finfish hatcheries as a source of live food for juvenile stages of commercial aquaculture species. The brine shrimp *Artemia*, especially its nauplii are considered as the best, sometimes the only live feed for the larval stages of shrimp and fishes. The main sources of this is from the harvest of natural population inhabiting at GSL, Utah, SFB, California and Chaplin lake, Canada. However, the quantum of supply is greatly influenced by environmental factors of these habits. The short supply of cysts leads to the high price, which in turns affect the cultivation of of shrimp and fishes. In India the growing demand for *Artemia*, continues to increase with the development and progress of aquaculture. In India, only parthenogenetic strain of *Artemia* has been reported to be present as natural population in the Sambhar, Didwana lakes in Rajasthan and salt pans of the Gulf of kutch, Gujarat, Vadala in Bomabay, and in Tamil Nadu state, Veppalodai near Tuticorin, Kareswar island off Tuticorin, Kelamakkan near Chennai and Vedaranyam sites. It is also interesting to note that these places of occurrence of *Artemia* are located in migratory route of flamingoes, which suggest that the birds are the possible agents for the distribution of same parthenogenetic strain in different parts of India.

The nutritional value of *Artemia* nauplii seem to be determined by their content of essential fatty acids. Wattanabe et al., (1980) classified different *Artemia* populations according to their fatty acid profile and suitability for feeding marine or freshwater organisms, in two broad groups (phenotypes). The so called marine types are characterized by a high percentage of eicosapentaenoic acid (EPA, 20:5n-3) and low percentage of linolenic acid (LNA; 18:3n-3). The freshwater types show low levels of EPA and high levels of LNA. The fatty acid composition of *Artemia* is considered to be more environmentally than genetically determined. (Bengston et al., 1991). Lavens et al., (1989) demonstrated that the fatty acid profiles of *Artemia* adults and the cysts they produce strongly reflect the fatty acid profile of their diet. These findings confirmed later on by Navarro and Amat (1992), Navarro et al., (1992, 1993), Triantaphyllidis G.V (1996). Most of the published works reported on fatty acid profile of *Artemia* strain cultured under laboratory level (Van stappen et al. 2003 Hanna et al., 2004). The biochemical divergence mainly caused by differences in the environment of *Artemia* population and the composition of food for the parental *Artemia* populations.

Hence to gain first hand knowledge, the field study was undertaken with the objectives (i) introducing inoculations of *Artemia* in traditional Indian solar salt bed to produce the biomass and cysts and (ii) to study their nutritional characteristics and relate to the diet available in the natural habitat.

2. PRESENT STATUS OF BRINE SHRIMP PRODUCTION

In early 80's, production of *Artemia* culture using Sanfracisco Bay strain have been initiated by Bharath Salt chemicals Industries, Gujarat, India. Tata Chemicals Ltd,

Gujarath achieved a production of dry cysts. In Tamil Nadu, Kelampakkam saltpan, conducted experiments on the *Artemia* cyst production using inorganic fertilizers. The author (Rahaman) have conducted an extensive field studies in different sites of Indian salt pans –Vedaranyam in Nagpattinam district, Kelambakkam, near Chennai, Tamil Nadu and Samphar lake saltworks, Rajasthan through multidisciplinary approach. The studies focused on biological factors and their relation to hydrological factors (Rahaman et. al., 2006 a), detailed account of biological management (2006 b & 1993a, b), dynamics of solar salt works (2006 c) sedimentation of salt works (2006 d.) All along the coastline, a vast area is under salt production and these solar salt pans offer excellent scope for carrying out *Artemia*, culture. Most of the infrastructure required for production is already available and *Artemia* form a valuable byproduct to salt farmers. However micro algae *Dunaliella salinas* are already available in these salt bands and also samphar salt lake, economic opportunity have been considered for commercial exploitation.

3. MATERIALS AND METHODS

Selection of site: The study was carried out of the DCW salt works, Sahupuram, Tamil Nadu, India. The present study in the extension of area that was selected earlier on coastal belt of India. The area consists of about 1400 acres of salt lands, and 140 acres of crystallization ponds. Out of this seven acres of land have been earmarked for *Artemia*, biomass and cysts production. The dyke construction, all parameters required for the study has been carefully implemented. Seven acres of condensation ponds have been divided in 14 ponds and pond No 2 is further divided with additional bunds in order to compare the productivity with other ponds.

Culture treatment: Imported Vietnam strain of *Artemia* was selected after personal communication with the authorities at *Artemia* Reference Center, Ghent University, Belgium. As elsewhere in the world, alternative *Artemia*, strains with better economic perspectives were sought and introduced. Since, Indian *Artemia* is parthenogietic and the cysts that it produces being big in size which are not suitable as larval feed. Further the Vietnam cysts are smaller and suitable to culture in natural climatic condition. Hence a trial study was conducted using Vietnam strain before it was introduced in to salt pans for commercial production. The salinity has been maintained at 60 ppt in the first pond and saline water is drained by gravitational flow to the other ponds. A few ponds were selected for cysts production in which salinity ranged between 80-120 ppt. Chemical fertilizer Urea, DAP, Potash and organic manure (chicken) were applied in different doses. During the adult stage, in addition to organic fertilizer, bacterial culture were added in the primary pond during pumping of the brine to enable the feed mixed and distributed to all the other ponds.

Under the field study, a parallel population of natural phytoplankton of halo tolerant algae *Dunaliella salina* was also maintained by adding inorganic fertilizers in the pond. Elimination of competing zooplanktons in the higher salinity coupled with cultivation of *Dunaliella salina* were biological managed. The continuous production and collections of the cysts for every 3rd day of culture period were done. The project was completed before the monsoon. The maximum biological productivity was achieved during summer. The biomass of *Artemia* was evaluated during the study. The first *Artemia* cysts were collected on the 18th day of its life cycle. On every third day, *Artemia* cysts were collected and feed was given thrice in a day. Cysts were collected during the evening time of the day, depending on the direction of the wind and the cysts were processed and stored in polyethylene bags. The rainfall data during the study period have been obtained by meteorological department. Samples for phytoplankton's were collected by filtering 50 liters of water through 10 micron plankton net, for Zooplankton and *Artemia* populations, the samples were taken with the help of 50 micron plankton net. All samples were preserved in 5% formalin solution. The biochemical analysis has been carried out taking

10 gm of cysts sample tested at Artemia Reference center, Ghent University and results are presented.

4. RESULTS AND DISCUSSION

Water quality parameters like temperature, dissolved oxygen content, pH, water salinity etc were measured. The total alkalinity, magnesium and calcium hardness, nutrients, primary productivity, chlorophyll content were measured. Phytoplankton and zooplankton communities were identified measured. The average pH 7-8.5., temperature ranging from 27°C to 40°C without any significant variation dissolved oxygen content.

Harvesting of cysts was started in higher salinity (90 ppt to 140 ppt) and biomass production was maintained in all ponds at 20-27 millions/pond. Cysts harvest has been increased to 32 kg./h during summer. Rate of production of live biomass was found to be 200 kg and 30 kg dry cyst /hectare.in 5 months. Cysts were semi processed in the field and later processed with fluidized bed drier retaining about 3 to 4% moisture content in the cysts.

Time	Hatching efficiency (nauplii/g;28 C)	Hatching percentage (28C)	Size
24hrs	227,578 n/g (15,233)	92.75% (0.74)	235.2 ± 1.3 micro meter

Content of fatty acids in first instar Nauplii

Fatty acids :20:5n-3	Area ;10.3%	12.0 mg/g dry wt. nauplii	Total fatty acids
22:6n-3	Area ;0.2%	0.2mg/g dry wt. nauplii	17 mg/gram

Artemia form a major, sometime the only consumer in hyper saline ecosystem. *Artemia* is a non-selective voracious plankton feeder and its density is largely determined by the availability of phytoplanktons. Our study gives the first hand information about the co-existence of various phyto elements and zoo plankters endemic to the present environments offered by solar salt works. The production of cysts and biomass is directly related to the environmental temperature and salinity of the culture media The *Artemia* population showed high levels of 20:5(n-3) Eicosapentaenoic acid content (EPA), which, to our knowledge, can be compared to the cysts from Madagascar –ANK populations cultured under laboratory conditions using mixed diet of the alga and yeast which shows the 24 mg/g under 17.3 area %, highest reported value in literature).

Generally feed conditions greatly determine the fatty acid profile of *Artemia* offspring. Increase of HUFA levels in *Artemia* offspring are aimed by few workers for commercial operations through intensive pond and tank cultures with different fertilization conditions. The most abundant fatty acids were found to be C16, C18 poly unsaturated fatty acids (PUFAs) isomers n-3, n-6. in different types of *Artemia* strains. The SFB strain from a temperate area showed a significant amount fatty acids of the type.18:2 n5, 18:3n3 and 18:4n3, .Oaxaca strain from southern Mexico had a 'marine' type profile characterized by 3% to 4% of the fatty acids C20:5n3 (eicosapentaenoic). The Great Salt Lake (GSL) strain cysts (*Artemia franciscana*) showed 4.1% C20:5, 0.1% 22:6 and 11.2%C18:4.

In our field studies the high levels of C20:5 omega 3 fatty acids under natural habitat conditions are possible only that significant amount of micro algal species halotolerant *Dunaliella salina* which is present under extreme environmental conditions. Since *Dunaliella salina* can accumulate extremely large amount of eicosapentatoic acid and docosahenoic acid when exposed to extreme conditions such as high light intensity, high salinity, extreme temperature and /or nutrient deprivation.

The very small size of cysts and nauplii of DCW salt work *Artemia* population together with exceptionally high levels in 20:5 omega 3 fatty acids make this source of particular

interest for commercial development. Because these fatty acids present in the *Artemia* cyst are in fact derived from the diet that *Artemia* feeds. Under laboratory conditions *Artemia* is fed with the encapsulated diets known to produce such higher content of fatty acids (Vos, 1984). However, such fatty acids productions in cost effective way is possible under natural environmental conditions. Earlier studies revealed that biodiversity of organisms in the sea water decreased as salinity and temperature increased due to environmental stress. As only one species of phytoplankton *Dunaliella salina* and the only one species of Zooplankton *Artemia* are able to tolerate at highest salinity possible is reflected in these current study. These results have been corroborated with earlier studies (Davis, 2000).

Dunaliella salina is able to synthesis beta carotene continuously day (photo synthesis) and night (dark respiration) under particular light intensity (UV range), high temperature (salt), and high salinity (magnesium content). Substratum in the salt pan offers nutrients, temperature retention and salt in the form of magnesium and hence photosynthetic activity has been triggered so as to accumulate the fatty acids.

Recently Lamers, 2008, reviewed the studies on regulatory mechanisms involved in beta carotene overproduction and explores & exploits the carotenoid accumulation of *Dunaliella salina* for cell-factory applications. In addition, studies claiming that carotenoids can prevent several human diseases especially, Eicosopentaenoic acid (EPA) plays an important role in mammals as an agent to prevent blood platelet aggregation. Furthermore, EPA plays a major role in modulating the biosynthesis of eicosanoids and in controlling the levels of blood lipids and lipoproteins. The clinical value of this fatty acid may be for the amelioration of atherogenesis and thrombogenesis. Studies on isolation and estimation of beta carotene and glycerol production of these strains are under progress.

5. CONCLUSIONS

The study on *Artemia* population at DCW solar salt work has shown that their commercial characteristics are quite good. The higher production of biomass and higher hatching efficiency and nutritional composition is great for commercial use. As the study has undertaken with proper biological management of solar salt pan, enrich the diet of *Artemia* by halo tolerant algae, offers excellent scope for getting nutrition live feed and quality salt production. At present algae as nutritional source of omega 3 PUFA's are expensive. Hence higher level of 20; 5n-3 omega fatty acid *Dunaliella salina* fed *Artemia* populations encourage and attractive for use by commercial food companies and health-care practitioners. The current study gains importance in the cultivation of brine algae under stress conditions without massive reduction in biomass content opens up new avenues for exploring this for commercial exploitation.

REFERENCES

1. Ahmed S.U. et al, 2000. *Study on the impact of fertilization on the production of Artemia (Cyst and Biomass) and salt in an integrated system from the solar salt works of Bangladesh*, *Biological Sciences*, 3(9): 1420-1423.
2. Bengtson D., Leger P. & Sorgeloos P., *Use of Artemia as food source*. In R.Browne, P.Sorgeloos & C.N.A.Trotman (eds), *Artemia Biology*. CRC Press, Boca Raton, Florida, USA: 255-285.1991.
3. Structure D.J.S., 2000. *Function and management of the biological systems for seasonalsolar saltworks*. *Global Nest: the international journal* 2, 217-226.
4. Davis J.S. *Biological & physical management information for commercial solar salt works*. *Proceeding of 1st international conference in the ecological importance of solar salt works*, 5-14. 2006
5. Triantaphyllidi G.V. et.al, 1996. *Characterization of two Artemia populations from Nambia and Madagascar: cytogenesis, biometry, hatching characteristics and fatty acid profiles*, *International study on Artemia*. LVI, Kluwer academic publishers, Hydrobiologia 335: 97-106.

6. Hanna H. et al, 2004. *Production of Lipids Rich in Omega 3 Fatty Acids from the Haotolerant Alga Dunaliella Salina*, *Biotechnology*, 3(1): 102-108.
7. Lamers P. et al. and the reference to their in, 2008. *Exploring and exploiting carotenoid accumulation in Dunaliella salina for cell-factory applications*. *Trends in Biotechnology*, Vol.26 No. 11.
8. Lavens P & Sorgeloos P., 2000. *The history, present status and prospects of the availability of Artemia cysts for aquaculture*. *Aquaculture* 181; 397-403.
9. Magna G.Y.G., Lpez J.C.P., Ortiz S.M.M. Roche E.D. and Davis J.S., 2005. *Recovery of a commercial salt works damaged by a hurricane-role of biological management* . *Proc.of 9th international conference on environmental science and technology*, 903-912.
10. Stephenson M. et al, 1989. *Fatty acid acylated proteins of the Halotolerant Alga Dunaliella Salina*, *Plant physiol*, 1989, Vol. 90.
11. Navarro J.C., Amar F. & Sargent J.R., 1993. *The lipids of the cysts of freshwater and marine-type Artemia*. *Aquaculture*, 109: 327-336.
12. Navarro J.C. & Amar F., 1992. *Effect of algal diets on the fatty acid composition of brine shrimp, Artemia sp cysts*. *Aquaculture*, 101: 223-227.
13. Rahamann A.A. et al., 2006. *Plankton Communities in Hypersaline Waters of Indian Solar Salt Works, Proceeding of the 1st International Conference on the Ecological Importance of Solar Saltworks*. Santorini Island, (October 20-22, 2006) Greece pp. 259.
14. Rahamann A.A. et al., 1991. *Hydrobiology of Artemia*, in Vedaranyam solar salt works, India, *In: proceedings of International Symposium on Biotechnology Symposium on Biotechnology of salt ponds*. Lanna chino (Ed) published by the salt Research Institute of Ministry of Light Industry PR. China pp. 283.
15. Rahamann A.A. et al., 2006. *Indian Solar Salt Works Production Processes and Chemical Composition of Salt, Proceeding of the 1st International Conference on the Ecological Importance of Solar Saltworks*. Santorini Island, Greece (October 20-22, 2006) pp. 249,
16. Rahamann A.A et.al *Sedimentology of Indian Solar Saltworks, Proceeding of the 1st International Conference on the Ecological Importance of Solar Salt works*. Santorini Island, Greece (October 20-22, 2006) pp. 229,
17. Rahamann A.A. et al., 2006. *Biological Management of Sambhar Lake Saltworks (Rajasthan – India), Proceeding of the 1st International Conference on the Ecological Importance of Solar Salt works*. Santorini Island, Greece, (October 20-22, 2006) pp. 199,
18. Rahamann A.A. et al., 2006. *Dynamics of Solar Salt works ecosystem in India, Proceeding of the 1st International Conference on the Ecological Importance of Solar Saltworks*. Santorini Island, Greece (October 20-22, 2006) pp. 122
19. Rahamann A.A. et al., 2006. *Sambhar Lake a Wetland – an assessment. Proceeding of the 1st International Conference on the Ecological Importance of solar salt works* Santorini Island Greece (October 20-22, 2006) pp. 38.
20. Rahamann A.A. et al, 1993. *Biological Management of Indian Solar Saltworks, Seventh symposium on Salt*, Elsevier Science Publishers, B.V.Amsterdam, Vol.I, pp.633-643.
21. Tackaert W. and Sorgeloos P., (1992). *The use of brine shrimp Artemia in biological management of solar saltworks*, Proc.7th International symp. On "Salt", Tokyo April 6-9.
22. Van Stappen G.L.Y. Sui N.H. Xin & Sorgeloos P., 2003. *Characterization of high-altitude Artemia populations from the Qinghai-Tiber Plateau*, PR China. *Hydrobiologia*, 500: 179-192.
23. Vos J., Vanhaecke P. & Sorgeloos P, 1984. *Quality evaluation of brine shrimp Artemia cysts produced in Asian Salt ponds*. *Hydrobiologia*, 108: 17-23.
24. Watanabe T., Oowa F., Kitajima C. & Fujita S., 1980. *Relationship between dietary value of brine shrimp unsaturated fatty acids*. *Bulletin of the Japanese Society of Scientific Fisheries*, 46: 35-41.