

-Review-

The Brine Shrimp *Artemia* and hypersaline environments microalgal composition: a mutual interaction

Fereidun Mohebbi

Iranian *Artemia* Research Center, Urmia, Iran

Abstract

Hypersaline environments are essential, integral and dynamic part of the biosphere. Their management and protection depend on an understanding of the influence of salinity on biological productivity and community structure. The aim of this study was to review the relationships between the two basic biological components of hypersaline environments (micro-algae and *Artemia*) to provide a better understanding the dynamics of these unique ecosystems. Algal composition as the main food source of *Artemia* determines *Artemia* growth, reproduction rates, brood size, density, lipid index and cysts yields. Furthermore, the reproduction mode of *Artemia* depends on food levels, so that at the low food levels the main reproduction going into cysts. On the other hand, seasonal fluctuations of algal abundance influence *Artemia* population in temperate large hypersaline lakes such as the Great Salt Lake (USA), Urmia Lake (Iran) and Mono Lake (USA). However, *Artemia* grazing pressure has significant effects on microalgal density.

Key Words: *Artemia*, hypersaline, microalgal composition, *Dunaliella*

Introduction

Hypersaline environments are important natural assets of considerable economic, ecological, scientific and natural value. These ecosystems span large areas worldwide, not only in salt production areas (solar salt works, salterns or Salinas) but also in natural lakes and lagoons, and in tidal ponds (Javor, 1989). Hypersaline environments of both marine and continental origin are essential, integral and dynamic part of the biosphere while the biogeochemical processes occurring in their unique ecosystems have considerable environmental, social and economic values (Shadrin, 2009). Interestingly, their unique physical and chemical characterization and distinctive biota set them apart from other aquatic ecosystems (Naceur et al., 2009). Hypersaline environments and biodiversity associated with

such environments are affected by both human activities and natural dynamics. For example, recent climatic changes have led to increasingly intense monsoon rains in some regions and to decreasing precipitation levels in others (Das Sarma, 2007). However, increased drought in arid and semi-arid regions of the world, which most of the inland hypersaline lakes are located on, had dramatic effects on these sensitive ecosystems. In fact, inland hypersaline lakes within closed hydrologic basins are subjected to natural and induced fluctuations in size and salt concentration over both short and long term intervals (Herbst and Blinn, 1998).

Hypersaline environments are generally defined as those containing salt concentrations in excess of sea water - 3.5% total dissolved salts (Das sarma and Arora, 2001). Velasco et al (2006) studied the

effects of salinity changes on the biotic communities in a Mediterranean hypersaline stream. They suggested that the number of taxa, Margalef's index and Shannon's diversity index decreased with increasing salinity. Their results supported the initial hypothesis that dilution causes an increase in richness and biotic diversity, but a reduction in abundance. In other words, as noted Borowitzka (1981): the more saline the water, the lower the species diversity and simpler the structure of the system. The number of studies that has been performed on *Artemia*-phytoplankton ecological relationships in natural biotopes is very limited; in most cases these studies refer to small-sized salt lakes, or to man-made salt ponds (e.g. Herbst, 2006; Tanner et al., 1999; Warnock et al., 2002). Persoone and Sorgeloos (1980) and Lenz (1987) reviewed literature data on the productivity of *Artemia* habitats in natural environments, both inland and coastal salt lakes.

Management and protection of these changing ecosystems depend upon an understanding of the influence of salinity on biological productivity and community structure. In other words, salinity changes both directly or indirectly affects the primary production which in turn may influence *Artemia*, as the major macrozooplankton of hypersaline waters. On the other hand, *Artemia* population is able to influence phytoplankton composition by feeding on them; therefore there is a continuous reciprocal interaction between *Artemia* and phytoplankton population in hypersaline environments. Although, abiotic parameters such as temperature, salinity etc. which form a background for biological activities and governed on these interactions, but since these ecological factors have been studied more frequently, so in this study we only focused on the interactions between *Artemia* and algae in hypersaline environments. However, when

necessary, the abiotic parameters have been argued. So, the objective of the present study was to attempt to provide a review based on the recent and previous studies on relationships between the two basic biological components in hypersaline environments i.e. microalgae and *Artemia* in order to provide a better understanding the dynamics of these unique ecosystems (Jellison and Melack, 1988).

Algal composition

Both prokaryotic and eukaryotic algae grow, and contribute to primary production in high salt concentration waters (Borowitzka, 1981).

Solar saltworks

The cyanobacterial mats of the solar lakes can be divided into two layers, the upper layer was dominated by coccoide forms such as *Aphanothece stagnina* (Sprengel) A.Braun, 1865 and *Synechococcus* sp., while the lower was dominated by filamentous species composed mainly of *Oscillatoria* and *Spirulina* species (Gamila, 1999). On the other hand, the microalgae of solar saltworks may be classified according to salinity tolerance, namely halobiont (salinity range 115 to 185 ppt) such as *Anacystis*, *Dunaliella* and *Spirulina*; halophilic (which can tolerate salinity range of 41 to 150 ppt) including *Lyngbya*, *Oscillatoria*, *Gloeocapsa*, *Synura*, *Amphora*, *Navicula* spp. *Nitzschia* spp. and *Suriella*; stenohaline which are abundant below 41 ppt, e.g. *Chaetoceros*, *Gyrosigma*, *Amphora marina* W.Smith, 1857, etc (Rahaman, 2006).

Williams (1981) suggested that the dissolved oxygen concentrations may well be important in determining salinity tolerance in halobionts and in certain halophiles. Dolapsakis et al (2005) concluded that in higher salinities *Dunaliella salina* (Dunal) Teodoresco, 1904, was able to increase its ratio to more than 50% and displace other

hypersaline microalgae, possibly as a better resource competitor.

Temperate lakes

In temperate hypersaline lakes, salinity as the major dominant parameter determines phytoplankton species composition and diversity. In most of these lakes, *Dunaliella* spp. mostly dominate other microalgae, due to their higher salinity tolerance. For example, in the Great Salt Lake it has been found that when salinity was near 200 g/l, the phytoplankton community was nearly a monoculture of *Dunaliella viridis* E.C.-Teodoresco, 1905, and nitrogen fixing cyanobacteria were absent or rare in the pelagic region of the lake (Rushforth and Felix, 1982), but when salinity decreased to about 50 g/l not only the density of phytoplankton increased, but also nitrogen fixing *Nodularia* sp. was detected in the lake. (Wurtsbaugh and Berry, 1990). Stephens and Gillespie (1976) demonstrated that when the lake salinity was higher and nitrogen-fixing cyanobacteria absent, nitrogen deficiency may have contributed to the summer decline in phytoplankton.

Wurtsbaugh and Gliwicz (2001) suggested that the pelagic community of phytoplankton in the Great Salt Lake during periods that *Artemia* were present as juveniles or adults (from Mars to June) was dominated by the unicellular green alga *Dunaliella viridis* E.C.Teodoresco, 1905, with more than 99% of the numbers of phytoplankton and between 84 and 99% of the biovolume. The densities of this alga were between 2800 and 4130 cells ml⁻¹. Other algae such as diatoms *Amphiprora* sp. and *Amphora* sp. contributed modestly to the biovolume on some dates. In the Great Salt Lake two genera of cyanobacteria namely *Aphanothece* and *Coccochloris* with the *Dunaliella* spp. along with the dozen or so bacterial species provide the food for grazing brine shrimp. Due to their thick silica cell walls, diatoms

though valuable, are tougher for the *Artemia* to digest.

The primary producers of benthic habitat in hypersaline lakes are dominated by cyanobacteria and some diatoms with the occasional green algae. The benthic primary producers are particularly active when the phytoplankton and brine shrimp density decline in the water column and resulting clear water allows light to penetrate to the bottom.

Microalgal composition of Urmia Lake is roughly similar to the phytoplankton in the Great Salt Lake, which consists predominantly of *Dunaliella*, with an important fraction of diatoms like *Nitzschia* and *Navicula* (Sorgeloos, 1997). However, other algae such as cyanobacteria have contributed in microalgal composition of Urmia Lake at more limited extent. For example, Mohebbi et al (2006) reported 3 cyanobacteria (*Anabaena*, *Oscillatoria* and *Synechococcus*) 2 green algae (*Dunaliella* and *Ankistrodesmus*) 11 diatoms (*Navicula*, *Nitzschia*, *Cyclotella*, *Symbella*, *Synedra*, *Pinnularia*, *Diatoma*, *Amphiprora*, *Surirella*, *Cymatopleura* and *Gyrosigma*). Reyahi et al (1994) observed 6 cyanobacteria (*Anabaena*, *Anacystis*, *Chroococcus*, *Lyngbya*, *Oscillatoria* and *Synechococcus*) 4 green algae (*Ankistrodesmus*, *Dunaliella*, *Monostroma* and *pandorina*) 2 diatoms (*Amphora* and *Navicula*). These variations may be related to limited and irregular sampling or increased salinity of the lake during recent years that likely has eliminated some non-tolerant species (Eimanifar and Mohebbi, 2007).

Quantitative analysis of chlorophyll a and algal density indicated that primary production in Urmia Lake is lower than that of its sister the Great Salt Lake and that *Dunaliella* is the dominant phytoplankton (more than 95% of the total phytoplankton in number) of both lakes (Van Stappen et al., 2001; Gliwicz et al., 1995 and Mohebbi et al., 2006).

Halotolerant algae of the genus *Dunaliella* are the most ubiquitous eukaryotic microorganisms in hypersaline environments which can survive even in saturated salt solutions. In Urmia Lake as the second largest hypersaline lake in the world *Dunaliella* was the dominant phytoplankton all seasons (Mohebbi et al., 2009). The much more salinity near saturation (>330 ppt) induced Urmia Lake *Dunaliella* to accumulate large amounts of beta-carotene in their chloroplasts (Mohebbi et al., unpublished data). This is a mechanism by which this alga is able to tolerate stress conditions such as high irradiance and salinity (López-Uc et al., 2009; Garcia et al., 2007).

More recently, a picoplanktonic (2-3 μ) green alga, *Picocystis salinarum* Lewin, 2000, was described from Mono Lake which dominates the phytoplankton community of the lake (Bruce et al., 2008).

Artemia and phytoplankton interactions

Brine shrimp in the genus *Artemia* are the dominant macrozooplankton present in many hypersaline environments (Wurtsbaugh and Gliwicz, 2001). This crustacean often dominates food web dynamics in hypersaline environments and its grazing activities control water clarity (Lenz, 1987; Wurtsbaugh, 1992) and consequently they are often introduced into salt production facilities to minimize algal blooms (Sorgeloos et al., 1986). The role of microalgae and *Artemia* in the production of high quality salt have been well documented (Davis, 1980).

Brine shrimp are non-selective filter feeders feeding on detritus scraped up from the bottom of the water column or on unicellular algae and other plankton higher up in the water column. Among other functions the rhythmic beating of the limbs pumps food and water through the median gully where the food is strained out by the limbs and retained in the food groove and later moved up to

the mouth, broken into smaller pieces or discarded (Borradale and Potts, 1967). Digestion begins with the mandibles reducing the size of food particles and with the addition of a sticky secretion from glands near the mouth. The secretion helps hold the bolus together while secretions all along the digestive tract add digestive enzymes (Brown 1960). The food is passed into the mouth, through esophagus and into the combined stomach and intestine. Nutrients are taken up all along the digestive tract before the waste is passed on through the anus. (Borradale and Potts, 1967).

It seems that algal composition as the main food source of *Artemia* in both natural habitat and culture media has significant effect on *Artemia* growth and reproduction rates. For example D'Agostino (1980) noted that growth of *Artemia* was influenced by both the species of phytoplankton in the diet and the culture media used to grow the phytoplankton. According to Wurtsbaugh and Gliwicz (2001), in the Great Salt Lake both the quality and quantity of the food source, combined with temperature likely influence growth rate and time to maturation of *Artemia*, as it does in other ectothermic animals (e.g. Wurtsbaugh and Cech, 1983). Savage and Knott (1998) studied the effects of limnological factors on parthenogenetic *Artemia* populations from Lake Hayward, western Australia. They suggested that the major mechanism controlling nauplius survival and recruitment of *Artemia* in Lake Hayward was food quality and quantity.

Low spring temperatures slow growth rates of *Artemia* in the Great Salt Lake (Wirick, 1972) and Mono Lake (Dana et al., 1995). In the summer, when perhaps lowered nutrient inputs and high grazing rates (Wurtsbaugh, 1992) decrease phytoplankton abundance, *Artemia* growth is slow (Dana et al., 1995). This occurs despite the fact that potential growth rates of well-fed animals are

maximal at midsummer lake temperatures of 25-28 ° C (Vanhaecke and Sorgeloos, 1980). Low brood sizes and lipid indices have also been related to the food limitation of the *Artemia* in the summer.

In large temperate lakes such as the Great Salt Lake, USA, Urmia Lake, Iran and Mono Lake, USA, where salinity differences are small and have little impact on the ecosystem and seasonal differences mainly imply temperature variations, generally *Artemia* has two generations per year. In fact, *Artemia* population is declining by lack of recruitment, increased predation and thermal death (Lenz, 1987). Therefore, large temperate lakes indicate explicit seasonal cycles with a high predictability in which fluctuations from one year to another are limited. The spring burst of egg (nauplii) production is well suited to saturate the environment with juveniles that can exploit the abundant phytoplankton food resource present then. As phytoplankton abundance decreased, nauplii survival declined greatly and the proportion of cysts produced increased significantly (Wurtsbaugh and Gliwicz, 2001). In the Great Salt Lake, early spring, when the primary producers have recovered from the previous year's grazing pressure, is the only time of high food levels. Therefore, this is the time of mass hatching from diapausing cysts and of intense reproduction in *Artemia* (Wurtsbaugh and Gliwicz, 2001).

Under low-food conditions, female *Artemia* have likely evolved mechanisms to produce cysts. According to Gliwicz et al., (unpublished data) in controlled laboratory experiments, *Artemia* produced 70-90% cysts when food levels were less than 2.5 µg chl a l⁻¹ and 60-70% nauplii when chl a levels were > 12 µg l⁻¹. Other factors such as temperature, salinity, photoperiod and brood number also contribute the mode of reproduction

(Berthelemy- Okazaki and Hedgecock, 1987), but food level appears to play a more important role.

On the other hand, primary production as food availability or food levels influences adult *Artemia* densities in various hypersaline lakes. The lower production in the Great Salt Lake supports peak summer adult densities of *Artemia* near 3 l⁻¹; whereas the more productive Mono Lake has densities of 6-8 l⁻¹ in the pelagic region (Conte et al., 1988).

High salinity of Urmia Lake during recent years up to saturation levels (about 350 ppt) has diminished *Artemia* population to less than 1 individual m⁻³ compared to 1 individual l⁻¹ during the high water levels (Mohammadi et al., 2009). It seems that increased salinity inhibits cyst hatching, limits availability of nutrients especially unicellular algae and causes high mortality due to excessive stressful condition. Additionally, cyst clutch sizes of Mono Lake *Artemia* are nearly double those observed in the Great Salt Lake (Dana et al., 1990) and cyst weights are equal to or greater than those in the Great Salt Lake (R.Jellison, Pers.comm).

Cysts yields in various hypersaline lakes and in different years can be influenced by primary production. For instance, in Mono Lake, *Artemia* produced 2.4 × 10⁶ to 5.1 × 10⁶ cysts m⁻² during different years, considerably more than the 6.5 × 10⁵ m⁻² produced in the Great Salt Lake in 1995 (Dana et al., 1990). This variation is likely because of a higher rate of primary production in Mono Lake (269-641 g C m⁻² yr⁻¹; Jellison and Melack, 1993) than in the Great Salt Lake (145 g C m⁻² yr⁻¹; Stephens and Gillespie, 1976).

Decreased salinity of the Great Salt Lake dramatically declined the *Artemia* abundance due to the appearance of new predator taxa in zooplankton assemblage (Wurtsbaugh and Berry, 1990). According to this study, small population of *Artemia* and other zooplankton were not able to

clear the water column, thus chlorophyll a levels remained high ($5\text{--}44 \mu\text{g l}^{-1}$) and secchi depths low (<2.2) throughout the year.

A single *Artemia* filters 240 mld^{-1} and therefore, at the average population density of 4 sub-adult and adult individuals per liter, this branchiopod is capable of filtering the entire lake volume once a day (Reeve, 1963). Thus the *Dunaliella* population density remains extremely low, and does the density of other green algae, diatoms and cyanobacteria that are able to reproduce fast enough to compensate for grazing losses.

According to Gliwicz (2003), in contrast to *Dunaliella* which is a typical euplanktonic species, many other taxa are not suspended in the lake water, but live in refuges where grazing losses are lower. These refuges are provided by the interiors of the long tubular setae of *Artemia* exoskeleton, which form the combs on the filtration appendages. The exoskeleton is shed at each of the 13 or 14 molts necessary for *Artemia* to attain maturity and large quantities float in the water. It was found that diverse algal-cyanobacteria community that colonize long filtration appendages of *Artemia*, represented up to 20% of the available food for adult *Artemia*, when the preferred free-swimming *Dunaliella* was at an extremely low density in the entire the Great Salt Lake southern arm. *Artemia* juveniles and nauplii can not readily access the algae colonizing discarded exoskeleton which induces higher survival in the older instars of *Artemia* than younger ones. This is evident from the seasonal changes in size distribution indicated by the densities of discrete size classes (Gliwicz et al., unpublished data).

As the food availability declines from June to November: the individual *Artemia* lipid index gradually decreased (Wurtsbaugh and Gliwicz, 2001); juvenile *Artemia* survival was held much lower than that of full grown adults; *Artemia*

mode of reproduction switched from cyst production to hatching eggs at low food levels in the lake. On the other hand, Gliwicz et al., (unpublished data) suggested that the *Artemia* body weight in the Great Salt Lake was considerably smaller than that of the Farmington Bay of the Great Salt Lake, where chlorophyll was much higher and *Artemia* less abundant due to lower salinity.

Artemia raised in laboratory in lake water without added food withheld eggs in egg sacks (Gliwicz et al., 1995). Female *Artemia* are prevented from allocating sufficient resources to reproduction below a threshold food concentration, which results in a long inter-brood interval as the clutches of eggs are withheld in the brood sacs. At the low food levels only 1 of 13 broods produced was ovoviviparous with the bulk of the reproduction going into cysts, and brood size were relatively small (15.6) (mean ± 7.3 ISD) eggs female $^{-1}$ day $^{-1}$; in contrast, in the high food levels 85% of broods were ovoviviparous and mean brood size was 53.6 (mean ± 24.8 ISD) eggs female $^{-1}$ day $^{-1}$ (Gliwicz et al., unpublished data).

Most of the first cohort *Artemia* was already adult by early May, but at the early June, the density of juveniles was dropped to one tenth of the expected value. Therefore, 90% of the second generation hatching from ovoviviparous eggs had died, evidently because food levels had declined from $25 \mu\text{g chl a l}^{-1}$ to less than $1 \mu\text{g chl a l}^{-1}$ in early June (Gliwicz et al., unpublished data).

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