Journal of Great Lakes Research 39 (2013) 47–55

Contents lists available at SciVerse ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr

Historic occurrence of parthenogenetic *Artemia* in Great Salt Lake, USA, as demonstrated by molecular analysis of field samples

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ARTICLE INFO

Article history:
Received 24 January 2012
Accepted 12 November 2012
Available online 20 January 2013

Communicated by Wendylee Stott

Keywords:
Artemia franciscana
Parthenogenetic *Artemia*
Great Salt Lake
Na/K-ATPase gene
HSP26 gene

ABSTRACT

Great Salt Lake (GSL), USA is the main source of the commercially important *Artemia franciscana* Kellogg (1906) cysts used in larviculture. Our objective was to document the presence of parthenogenetic *Artemia* in GSL analysing a series of non-commercial samples harvested over the period 1997–2005. Laboratory cultures suggested that sex ratios were skewed in some years. Species-specific restriction fragment length polymorphisms in the exon-7 of the Na/K-ATPase α-1 subunit nuclear gene and of a fragment of exon-2 of the heat shock protein HSP26 gene were used to identify samples of individual adults and pooled cysts. Additionally, denaturing gradient gel electrophoresis using the Na/K-ATPase marker and individual adults was used because of its greater power for detecting different alleles. Finally, the exon of the Na/K-ATPase α-1 subunit was sequenced in selected individuals to validate the results. All results indicated that there were parthenogenetic *Artemia* in the samples from the period 2000 to 2002. Our data do not provide evidence on the autochthonous or allochthonous nature of this population, although an anthropogenic origin seems most likely. The transitory character of the incidence of parthenogenetic *Artemia* can be linked to unusual environmental conditions in the lake around the turn of the century. The subsequent disappearance of the parthenogenetic population would then be due to the competition with the more productive *A. franciscana* population as conditions returned back to normal. A systematic genetic study of the GSL *Artemia* population is recommended as it may provide valuable complementary information about population changes undetected in traditional monitoring programmes.

Introduction

The brine shrimp *Artemia* (Crustacea, Anostraca) is a zooplanktonic organism found worldwide in hypersaline habitats such as inland salt lakes, coastal salt pans and man-managed saltworks. *Artemia* is well adapted to these environments, due to its unique osmoregulatory capability, its capacity to synthesize highly efficient haemoglobins and its ability to produce dormant ("diapausing") cysts when ambient conditions such as salinity and temperature become extremely unfavourable. Upon termination of the diapause stage, the cysts hatch into free-swimming nauplii when conditions are favourable.

The genus *Artemia* consists of a number of obligately sexually reproducing species and numerous obligately parthenogenetic populations. Because there are few visible morphological differences between the various species of the genus, species are increasingly being identified using species specific sequences in nuclear and mitochondrial genomes. Seven sexual species and many parthenogenetic populations are currently recognized (Gajardo et al., 2002). Parthenogenetic populations are only found naturally in Europe, Asia, Africa and Australia, where they sometimes co-exist in the same environment with the various sexual species found in those continents. Parthenogenetic populations generally occupy different niches than their sexual counterparts, depending on the preference of the local populations for specific abiotic conditions. In Lake Urmia, Iran, for example, parthenogenetic *Artemia* are restricted to shallow coastal areas with extreme seasonal salinity fluctuations, whereas the local sexual species *Artemia urmiana* Günther (1890) is found in offshore areas of the lake where fluctuations are limited (Agh et al., 2007). In coastal salt ponds in Spain, the sexual species *Artemia salina* Leach (1819) dominates in the cooler winter months, whereas the co-occurring parthenogenetic population dominates in summer (Amat, 1983). Although these parthenogenetic populations are sometimes grouped for convenience under the binomen *Artemia*

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http://dx.doi.org/10.1016/j.jglr.2012.12.017

parthenogenetica Barigozzi (1974), there is unanimity among *Artemia* taxonomists to refer to them merely as 'parthenogenetic strains' or 'parthenogenetic populations' (Abatzopoulos et al., 2002). In North and South America on the other hand, all brine shrimp populations belong to the sexual species *Artemia franciscana* Kellogg (1906) (Van Stappen, 2002), with the exception of the extreme south of South America, where the sexual species *Artemia persimilis* Piccinelli and Prosdocimi (1968) occurs.

Nauplii of *Artemia* are the most widely used live food item in the larviculture of fish and shellfish. Though *Artemia* strains reaching the global cyst market nowadays are quite diverse in geographic origin, including parthenogenetic resources from south Siberia, Central Asia and inland China, the main source of *Artemia* cysts is the *A. franciscana* population from Great Salt Lake (GSL), covering more than 90% of the world market (Dhont and Sorgeloos, 2002). Great Salt Lake is a desertic hypersaline terminal lake with a surface area in the range 4000–6000 km^2, located in the Great Basin, Utah, USA (40°40′N, 112°20′W). Like other terminal lakes, depth, volume and salinity fluctuate widely in response to climatic cycles that determine rates of evaporation and precipitation (Wurtsbaugh and Smith Berry, 1990). Over the past decades, salinity in Gilbert Bay, the most important part of the lake for *Artemia* production, has fluctuated in the range 60–150 g/L (Dhont and Sorgeloos, 2002). Due to their importance both from an ecological and an economic point of view, the lake and its *Artemia* population have been the focus of many ecological studies over the past few decades (e.g. Naftz et al., 2008; Nielson and Bowen, 2010; Stephens, 1990, 1998; Stephens and Gillespie, 1976; Wurtsbaugh and Gliwicz, 2001). Annual harvests from GSL vary considerably, both in terms of quality and quantity, as a result of short and long-term fluctuations of environmental conditions. These fluctuations influence the productivity of the *A. franciscana* population both directly and indirectly, through phytoplankton abundance and species composition (Lavens and Sorgeloos, 2000).

It is generally recognized that only the sexually reproducing *A. franciscana* is present in Great Salt Lake (Abatzopoulos et al., 2002). Although there is no evidence thus far for coexistence with a local parthenogenetic strain, a recent study has documented the presence of parthenogenetic individuals in commercial GSL samples (Campos-Ramos et al., 2003). Whether this was due to the mixed nature of the harvested population or to mixing of batches of different populations through the industrial processing line is not known. In the Mediterranean Basin and in Asia, there is increasing evidence for the human impact on *Artemia* species distribution through the deliberate and non-deliberate inoculation of the allochthonous *A. franciscana* in hypersaline coastal environments (Amat et al., 2007; Green et al., 2005; Van Stappen et al., 2007). In contrast, no similar case of introduction has been reported for the Americas to date. In view of the importance of the GSL resource for the global *Artemia* cyst market, knowledge on the species status of its population is essential as different *Artemia* species may have different productivity characteristics. For this purpose, commercial samples are generally not a suitable study object unless the researcher has absolute certainty about their purity and geographical background. The objective of our study was therefore to confirm or refute the presence of parthenogenetic *Artemia* in GSL by analysing non-commercial field samples, which had been harvested from GSL over a period of 9 years (1997–2005) and were stored in the cyst bank of the Laboratory of Aquaculture & Artemia Reference Center (ARC). By analysing a time series of samples, we aimed to obtain a more representative view and to detect possible temporal changes in species composition. Older samples, available in the ARC cyst bank, had been found to be non-hatchable and more recent field samples of non-commercial origin were not available to us. As a first screening, a laboratory culture test was run to detect the parthenogenetic strain through sex ratio data. Enzymatic digestion revealing restriction fragment length polymorphism (RFLP) in markers having a literature record of discriminatory power between *Artemia* species was used on both pooled cysts and individual adults: polymorphism of exon-7 of the Na/K-ATPase α-1 subunit nuclear gene has been demonstrated by Manaffar et al. (2011), allowing to discriminate between parthenogenetic and sexual *Artemia*, and therefore this fragment was chosen as marker A fragment of exon-2 of the heat shock protein HSP26 gene was also used, based on its *A. franciscana*-specific polymorphism as demonstrated by Beristain et al. (2010) who used it to discriminate between *A. franciscana* and *A. persimilis*. The nucleotide sequence of these genes, as found in the National Center for Biotechnology Information (NCBI) database for *A. franciscana* and parthenogenetic *Artemia*, was searched for restriction enzyme cleavage sites diagnostic for either species. For confirmation, the RFLP technique was combined with denaturing gradient gel electrophoresis (DGGE) on individual adults because of its power to detect genetic variability as small as single base substitutions, undetectable by the RFLP technique (Dooms et al., 2007; Myers et al., 1987). For final validation we sequenced the bands produced by DGGE.

Material and methods

Artemia cyst samples

Nine non-commercial cyst samples of *A. franciscana*, collected from GSL between 1997 and 2005, and stored at −20 °C in the cyst bank of the Laboratory of Aquaculture & Artemia Reference Center (ARC), Ghent University, were included in this study (Table 1). Pooled cyst samples and individual adults, grown out of the hatched nauplii, were used for DNA extraction.

Hatching and culture of Artemia

Cysts were hatched in diluted artificial Instant Ocean® seawater of 5 g/L salinity in order to enhance hatching (Lavens and Sorgeloos, 1996). For each sample, 80 mg of cysts was incubated in 50 mL Falcon tubes containing 40 mL of medium. The tubes were provided with aeration by inserting a pipette through the pierced lid and kept at room temperature (20–22 °C) with permanent illumination using fluorescent lights. Hatching percentage was calculated after 24 and 48 h using standard methodology (Lavens and Sorgeloos, 1996). Adult individuals were obtained by transferring 60 nauplii of each sample to a 1 L cilindroconical glass bottle containing 400 mL of 35 g/L artificial sea water. Aeration was provided with a pipette through the pierced lid of the bottles; *Artemia* were fed ad libitum with *Tetraselmis suecica* algae and the culture water was renewed every 3 to 4 days and the surviving animals were counted.

Table 1
A. franciscana samples from Great Salt Lake used for molecular analysis, in chronological order of harvesting year, with corresponding ARC (Laboratory of Aquaculture & *Artemia* Reference Center) cyst bank number and codes used for the pooled cyst samples and individual adults from which DNA was extracted.

Year of harvest	ARC cyst bank number	Pooled cysts code	Individual adults code	
			Males	females
1997	1685	C_1	$M_1$1–3	$F_1$1–3
1998	1684	C_2	$M_2$1–3	$F_2$1–3
1999	1508	C_3	*	*
1999	1683	C_4	$M_4$1,2	$F_4$1–3
2000	1521	C_5	**	$F_5$1–3
2001	1681	C_6	$M_6$1–3	$F_6$1–3
2002	1680	C_7	***	***
2003	1679	C_8	$M_8$1–3	$F_8$1–3
2005	1677	C_9	$M_9$1–3	$F_9$1–3

* Only one female and one male survived to maturity, which was considered insufficiently representative; hence these individuals were not used for DNA extraction.
** No adult males were found when sexual maturity was reached.
*** DNA was not extracted from adults because of sample damage before DNA extraction.