Aquatic Toxicology 161 (2015) 208–220

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Aquatic pollution may favor the success of the invasive species *A. franciscana*

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ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 11 February 2015
Accepted 17 February 2015
Available online 19 February 2015

Keywords:
Artemia
Invasion
Toxicity
Chlorpyrifos
Life tables
AChE

ABSTRACT

The genus *Artemia* consists of several bisexual and parthenogenetic sibling species. One of them, *A. franciscana*, originally restricted to the New World, becomes invasive when introduced into ecosystems out of its natural range of distribution. Invasiveness is anthropically favored by the use of cryptobiotic eggs in the aquaculture and pet trade. The mechanisms of out-competition of the autochthonous *Artemia* by the invader are still poorly understood. Ecological fitness may play a pivotal role, but other underlying biotic and abiotic factors may contribute. Since the presence of toxicants in hypersaline aquatic ecosystems has been documented, our aim here is to study the potential role of an organophosphate pesticide, chlorpyrifos, in a congeneric mechanism of competition between the bisexual *A. franciscana* (AF), and one of the Old World parthenogenetic siblings, *A. parthenogenetica* (PD). For this purpose we carried out life table experiments with both species, under different concentrations of the toxicant (0.1, 1 and 5 μg/l), and analyzed the cholinesterase inhibition at different developmental stages. The results evidence that both, AF and PD, showed an elevated tolerance to high ranges of chlorpyrifos, but AF survived better and its fecundity was less affected by the exposure to the pesticide than that of PD. The higher fecundity of AF is a selective advantage in colonization processes leading to its establishment as NIS. Besides, under the potential selective pressure of abiotic factors, such as the presence of toxicants, its higher resistance in terms of survival and biological fitness also indicates out-competitive advantages.

1. Introduction

Aquatic hypersaline ecosystems (salt lakes, marine and inland solar saltworks, etc...) show very simple trophic structures and very low biodiversity for their conspicuous invertebrate native fauna (Lenz and Browne, 1991). Most representative of these invertebrates are the species of the cosmopolitan brine shrimp genus *Artemia* (Crustacea, Branchiopoda, Anostraca), that show very strong adaptability to diverse hypersaline environments, mediated by a broad tolerance to extremely variable salinities, ionic brine composition, temperatures and oxygen concentrations (Triantaphyllidis et al., 1998; Amat et al., 2005).

The genus *Artemia* consists of several sibling species. Six bisexual species have been recognized, two of them restricted to the New World: *A. franciscana* and *A. persimilis*. The former is the most abundant, and its cysts are commercially available as a natural resource for aquaculture. The other four bisexual species: *A. salina*, *A.*, *urmiana*, *A. sinica* and *A. tibetiana*, together with a heterogeneous group of obligate parthenogenetic strains recognized as *A. parthenogenetica* (Muñoz et al., 2010), are restricted to the old world. In the Mediterranean basin, southern Europe and North Africa, the biodiversity of the genus is reduced to the native sexual *A. salina*, which sometimes coexists with a variety of diploid and tetraploid parthenogens, with diploids being the most abundant (Amat et al., 1995). Investigations about the comparative biological fitness among different species of the genus, suggest greater physiological, lifespan, reproductive trait and population dynamics differences related to environmental conditions, with important intrinsic variability associated with salinity and temperature (Browne et al., 1988; Browne and Halanych, 1989; Barata et al., 1996; Browne and Wanigasekera, 2000; Amat et al., 2007; Pinto et al., 2014).

Aside from being a cosmopolitan organism, a wide array of intrinsic characteristics, such as the relatively easy rearing and maintenance of experimental populations under different

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http://dx.doi.org/10.1016/j.aquatox.2015.02.008
0166-445X/

temperatures and salinities, their resistance to manipulation, short life-cycle, large offspring production, etc., have allowed the brine shrimp *Artemia* to become an important tool organism in several scientific disciplines, such as ecology, physiology, genetics and aquatic ecotoxicology. Despite some criticisms having been raised against the inclusion of *Artemia* as a marine-water test organism, because of its absence in marine ecosystems and its presumed lack of sensitivity to chemical exposure due to its conspicuous resistance to extreme salinity (Persoone et al., 1987), toxicity bioassays – particularly dealing with marine and brackish water ecosystems – have been proposed using the genus as a test organism as broadly reviewed by Nunes et al. (2006). However, many ecotoxicological studies using brine shrimp *Artemia* as a test organism do not take into account the biodiversity of the genus. Most often, species or strains of different origin are neither properly identified nor cited in the materials and methods section, or worse, individuals obtained from commercial cysts are mistakenly reported as *A. salina* in reference to the holotype nomenclature, when the vast majority of commercial cysts (if not all) belong either to parthenogenetic strains or most likely to the American *A. franciscana* (Crisine et al., 1994; Barahona-Gomariz et al., 1994; Venkateswara Rao et al., 2007). So, the current picture is that despite the availability of a substantial amount of information dealing with fundamental aspects of ecotoxicity testing (Nunes et al., 2006), it is possible to state that most of it was produced using *A. franciscana*, with a generalised lack of data about the proper geographical localization of the origin and characterization of the cysts, species or populations used in the bioassays. Work performed by Browne (1980), Sarabia et al. (2002) and Varó et al. (1998) showed consistent distinct patterns of response to metals or pesticides, according to different species or strains for testing purposes.

On more empirical grounds, *Artemia* cysts, i.e., its cryptobiotic eggs, are widely used in aquaculture to produce nauplii that are used as live food for rearing early stages of molluscs, crustaceans and fish. The majority of commercial cysts rely on their natural occurrence in the Great Salt Lake and San Francisco Bay saltworks (United States) (Lavens and Sorgeloos, 2000), and this has resulted in the widespread distribution of *A. franciscana* all over the world. For example, field studies carried out in the western Mediterranean region from the early 1980s report the occurrence of exotic *A. franciscana* populations (Amat et al., 2005). Other anthropogenic activities such as pet trade, or saltwork operations have also contributed to the expansion and dispersion of this American native species, which has become an invasive pest that out-competes and eradicates native *A. salina* and *A. parthenogenetica* populations in wetlands and saltworks (Amat et al., 2005; Green et al., 2005), acting as an alien or non indigenous species (NIS, Piola and Johnston, 2008). The mechanisms of eradication and out-competition of the autochthonous *Artemia* by the invader *A. franciscana* are, however, still poorly understood. It seems clear that the highest ecological fitness of the invader plays a pivotal role, but other less explored, underlying biotic and abiotic (i.e., sensitivity to toxicants) processes, may be affecting the outcome.

This study starts from the evidence of the presence of toxicants in the brines of a hypersaline ecosystem, the river Ebro delta salterns, in which the invader *A. franciscana* has replaced the original autochthonous *A. parthenogenetica* populations (Serrano et al., 2012). It aimed at studying the potential role played by an organophosphate pesticide namely chlorpyrifos in a congeneric competition mechanism leading to the establishment of this NIS and to the disappearance of the native populations. For this purpose, life table experiments and further demographic analyses were carried out with both species, under different concentrations of the toxicant (0.1, 1 and 5 μg/l), and the cholinesterase inhibition at different developmental stages was analyzed.

2. Materials and methods

2.1. Experimental organisms and conditions

Two different *Artemia* populations were used in this study: *A. franciscana*, a non-indigenous invasive species in Europe original from America (hereafter indicated as AF), and the native diploid *A. parthenogenetica* (hereafter indicated as PD). PD originates from La Mata salt lagoon (38°02′08′′N 0°42′30′′W) in Torrevieja (Alicante, Spain). AF was introduced in “La Trinitat” saltworks at Alfaques Bay (40°34′58′′N 0°40′48′′E) in the river Ebro delta (Tarragona, Spain), and it practically eradicated the parthenogenetic *Artemia* native populations in this location (Amat et al., 2005).

Original brine shrimp cysts collected in 1988 for La Mata PD and in 2008 for “La Trinitat” AF, and kept at the “Instituto de Acuicultura Torre de la Sal” (IATS) cyst bank, were used. These cysts were hatched in seawater (35 g/l) at 28 °C under 24 h light photoperiod (1500–2000 lux, using fluorescent lights) and aeration by air bubbling. Newly hatched nauplii were separated from their empty floating shells and any remaining unhatched cyst was discarded.

Batches of 150 newly hatched nauplii per treatment were separated in order to have a sufficient number of individuals of the same age for life table experiments. From each batch, a total of 5 replicates of 30 early metanauplii were separated and directly transferred to polypropylene corning tubes filled with 50 ml of experimental solution (see below for details). The cultures were kept under conditions of constant temperature, salinity and photoperiod in a thermostatic chamber (24 °C, 75–80 g/l, 12 h:12 h light:darkness), and fed on a mixture of the microalgae *Dunaliella salina* and *Tetraselmis suecica*, which is the staple diet for the maintenance of *Artemia* cultures at IATS. After one week, animals were transferred to 150 ml jars filled with 100 ml of experimental solution. Complete medium renewal was performed every 2–3 days in order to maintain the exposure conditions (concentration of chlorpyrifos in the water and food) as constant as possible (Varó et al., 2000) and to preserve individuals from excessive handling. Previous determinations showed that after 48–72 h actual concentrations of chlorpyrifos in water were less than 10% from nominal values (Varó et al., 2000; Varó et al., 2000) The cultures were maintained in these conditions until the females showed the first signs of vitellogenesis (Sarabia et al., 2008).

2.2. Chemicals and experimental solutions

Prior to carrying out this study, brine and living *Artemia* biomass samples, collected seasonally over one year (2007) in “La Trinitat” saltworks, were analyzed in the Research Institute for Pesticides and Water, University Jaume I in Castellón (Spain). A GC/TOF MS-based method was applied in search of organochlorine and organophosphorous contaminants (Serrano et al., 2011, 2012). The pesticide chlorpyrifos was commonly detected in brine and brine shrimp samples. This insecticide is usually applied to protect agricultural crops, mainly rice fields, spread throughout the river Ebro delta (Claver et al., 2006) reaching the Alfaques Bay from where seawater is pumped to be evaporated in “La Trinitat” saltworks for marine salt production.

Chlorpyrifos was obtained from Dr. Ehrenstorfer Reference Materials (Germany). Several stock solutions were prepared by dissolving chlorpyrifos in acetone given the low solubility of chlorpyrifos in saline waters (Varó et al., 2000). The experimental solutions were obtained by serial dilutions of chlorpyrifos stock in filtered (0.45 μm) brine (75–80 g/l). Three different sublethal chlorpyrifos concentrations were tested: 0.1, 1 and 5 μg/l. Two controls were also included. A first control group was exposed to clean brine (C), and a second one was exposed to the same amount of solvent (5 μg/l acetone, CA) as in the highest concentration tested.