

## Biodiversity loss in the genus *Artemia* in the Western Mediterranean Region

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### ABSTRACT

#### Biodiversity loss in the genus *Artemia* in the Western Mediterranean Region

Local populations extinctions depend on intrinsic factors related to the biology of species, as well as on direct threats of stochastic and/or deterministic type. The species of the genus *Artemia* (Branchiopoda, Anostraca), conspicuous inhabitants of hypersaline ecosystems, are suffering an important biodiversity loss. Their persistence is dramatically affected by deterministic factors threatening their biotopes. Among these deterministic factors the loss of habitats and the introduction of exotic invasive species are the most relevant. This paper aims to summarize the information available on: a) the abandonment of solar salterns in the Western Mediterranean region; b) the present distribution of the exotic invasive species *Artemia franciscana* populations in Spain, Portugal, Italy and France; c) several reproductive parameters obtained from the study of life tables, performed for several autochthonous species and strains and the invasive species. These traits, linked to the quantitative and qualitative offspring output, allow an experimental approach to the understanding of the fitness superiority of the invasive species, as well as to the competitive displacement of the autochthonous *Artemia* species in the Western Mediterranean region.

**Key words:** *Artemia*, loss of habitats, invasive species, fitness, competition, displacement, Western Mediterranean.

### RESUMEN

#### *Pérdida de biodiversidad en el género Artemia en la región del Mediterráneo Occidental*

La extinción de poblaciones locales depende de factores intrínsecos que tienen que ver con la biología de las especies, así como de amenazas directas de tipo estocástico o determinístico. Las especies del género *Artemia* (Branchiopoda, Anostraca), conspicuos pobladores de humedales hipersalinos, están sufriendo una importante pérdida en biodiversidad. Su persistencia se ve dramáticamente afectada por factores determinísticos que amenazan a sus biotopos. De entre estos factores, la pérdida de hábitats y la introducción de especies exóticas invasoras son los más relevantes. Este trabajo pretende resumir la información disponible sobre: a) el abandono de salinas solares en la región del Mediterráneo Occidental; b) la distribución actual de poblaciones de la especie exótica invasora *Artemia franciscana* en España, Portugal, Italia y Francia; c) varios parámetros reproductivos obtenidos del estudio de tablas de vida, llevadas a cabo para varias especies y estirpes autóctonas y la especie invasora. Estos aspectos cuantitativos y cualitativos de la descendencia permiten una aproximación experimental a la comprensión de la superior eficacia biológica de la especie invasora, así como del desplazamiento por competencia de las especies autóctonas de *Artemia* en la región del Mediterráneo Occidental.

**Palabras clave:** *Artemia*, pérdida de hábitats, especies invasoras, eficacia biológica, competencia, desplazamiento, Mediterráneo Occidental.

## INTRODUCTION

The impact of invasive species on the ecosystems, communities and native species is known since several decades ago (Elton, 1958; Lodge, 1993; Simberloff, 1996). The level of knowledge and understanding of the mechanisms leading to the success of the invasive is far to provide neither criteria to predict the threat of invasions nor how to act or to manage ecosystems already invaded. The understanding of invasive mechanisms compel to an exhaustive comprehension of the interactions triggered between the exotic and the native species, since their fate becomes always rather more complex (Wootton, 1994; Grosholz & Ruiz, 1996; Byers, 2000).

Invasion success and impacts depend on the competitive and trophic interactions to arise in the environment where invasive and analog native species meet, but the knowledge of these interactions is only possible when the invasion has already happened. If the exotic species behaves openly as invasive, the prospects ahead are probably irreversible (Herbold & Moyle, 1986). The research on invasive species offers singular opportunities for the understanding of population's biology, due to the evidence that their evolution proceeds faster than usual in natural environments (Sakai *et al.*, 2001).

The introduction of exotic invasive species where congeneric native species exist leads to the extinction of the latter in the long term. Invasions and the loss of fit environments for the development of autochthonous species are in the basis of the global biodiversity damage. Biodiversity, a dynamic process shaped by time and the interaction of natural processes, biotic and abiotic, is threatened by the present globalization phenomenon, together with alien species introduction, hybridizations, displacements and extinctions of autochthonous species (Sakai *et al.*, 2001; Mooney & Cleland, 2001; Jiménez Pérez, 2005).

Aquatic ecosystems are also suffering from these impacts markedly. Freshwater or continental environments, like the Ebro River that shows the presence of zebra mussel (*Dreissena polymorpha*), or the marine habitats displaying macroalgae invasions, like *Caulerpa taxifolia* in the Western

Mediterranean, serve as examples in our latitudes. Hypersaline aquatic environments, like coastal and epicontinental lagoons or exploited solar salterns, characterized by a high productivity and low diversity, are not free of these impacts. In these environments, one of their most conspicuous inhabitant, the brine shrimp *Artemia*, is probably the most dangerously threatened.

The genus *Artemia* comprises a group of six bisexual species and a variety of parthenogenetic strains of diverse ploidy. Two of these bisexual species are autochthonous from America. *Artemia franciscana* is probably the most abundant, spread all around the American continent (Vanhaecke *et al.*, 1987; Hontoria & Amat, 1992b; Triantaphyllidis *et al.*, 1998; Abatzopoulos *et al.*, 2002). In the American South Cone *A. persimilis* is nearly exclusive (Cohen *et al.*, 1999; Amat *et al.*, 2004; Gajardo *et al.*, 2004). In the Mediterranean basin a bisexual species *A. salina* occurs together with two parthenogenetic strains, one diploid and another tetraploid. Several taxa may occur in sympatry, developing concurrently or seasonally overlapping populations along the year (Amat, 1983; Lenz & Browne, 1991; Hontoria & Amat, 1992a; Amat *et al.*, 1995). These strains became common in all the countries of the Mediterranean area, in Southern Europe, as well as in North Africa, in accordance to their phenotypic plasticity and the fitness traits of their life history, determined by climatic parameters inherent to their conspicuously changing hypersaline environments (Abatzopoulos *et al.*, 2002). The other Old World species are distributed in Asia, with the exclusive presence of *A. urmiana* in lake Urmia (Iran), *A. sinica* in China and neighboring areas, and *A. tibetiana* present in salt lakes of the Tibetan plateau. Parthenogenetic strains, mainly diploid, are also abundant in Asia, coexisting or not with the bisexuals.

The biodiversity of *Artemia* populations in the Mediterranean basin has suffered a great change after the first recording of *A. franciscana* specimens. This event was initially stated in Portugal (Hontoria *et al.*, 1987) and later in France (Thiery & Robert, 1992). Recent reports demonstrate this invasion in Spain and Morocco (Amat *et al.*, 2005; Green *et al.*, 2005). Today the

invasive species is present in the Italian salterns from Margherita di Savoia (Mura *et al.*, 2004), and just recorded in La Trinidad salterns (Alfaques Bay, Tarragona, Spain) as described in this work.

Several attributes of biological fitness characterizing invasive species are known after data from Ehrlich (1984), Lodge (1993), Shigesada & Kawasaki (1997) and McMahon (2002). However, their invasive potential becomes evident when fitness mechanisms are associated with human activities enabling their arrival or introduction to new ecosystems. In the case of *Artemia*, the important role played by this crustacean in the development of salt exploitation in big industrial solar salterns, or in the modern marine aquacultural technologies, likely provoked the initial introductions of *A. franciscana* evidencing its status of exotic and invasive species.

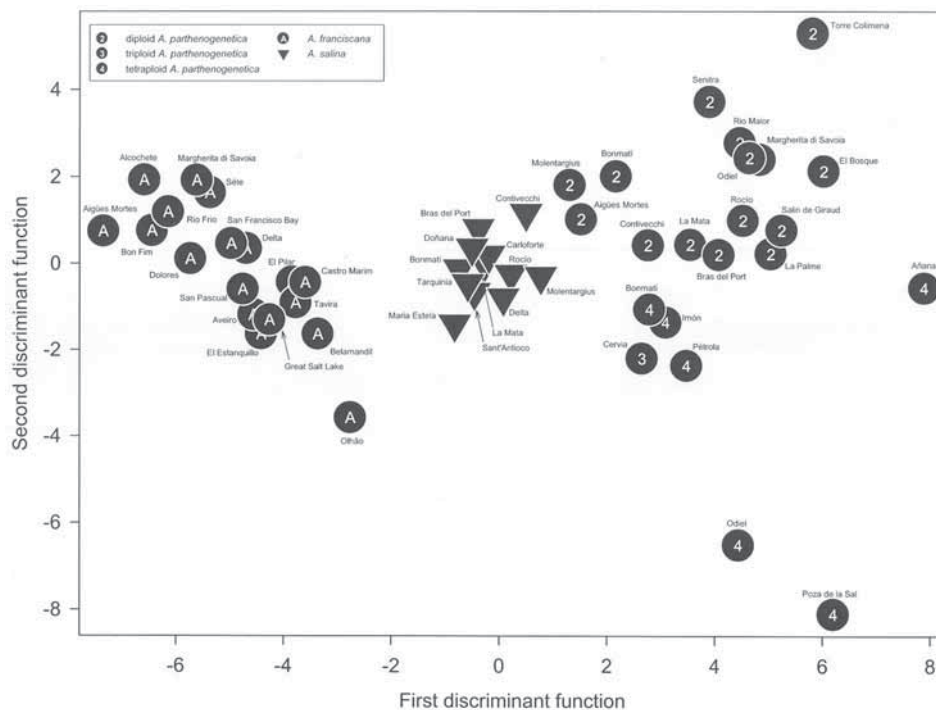
Once acknowledged the important threats on biodiversity defined by some deterministic factors (Jiménez Pérez, 2005) like the introduction of exotic invasive species and the fragmentation

or loss of habitats, and by some stochastic factors, basically demographic, bound to biological fitness, this work aims to a) summarize the loss of hypersaline environments essential for *Artemia* populations, mainly focusing on solar salterns abandonment in the Western Mediterranean region during the last decades, b) the identification of new invasive *Artemia franciscana* populations appeared since Amat *et al.*, (2004) and c) compare reproductive parameters for some autochthonous and invasive *Artemia* strains developed in the laboratory under static conditions.

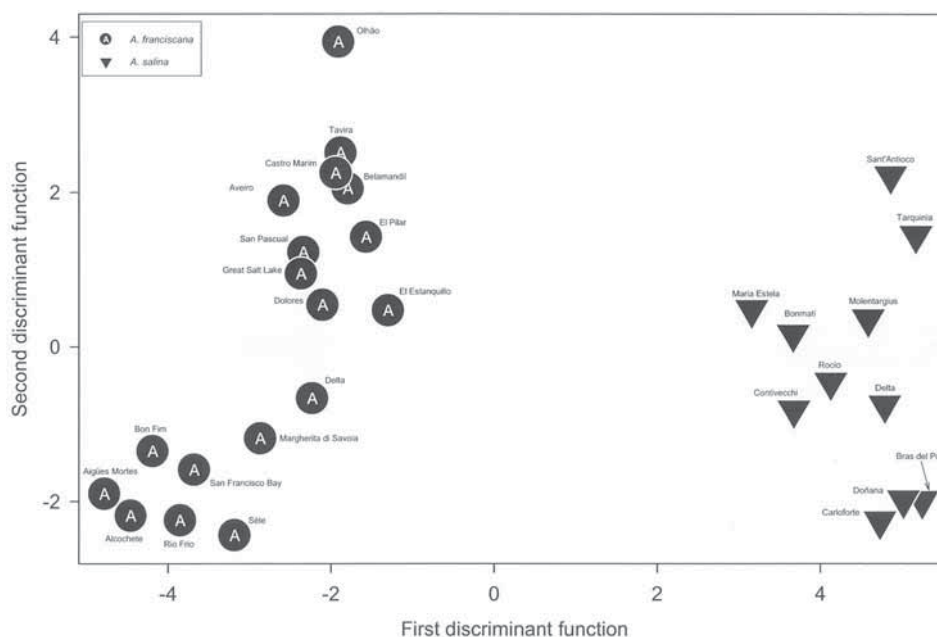
## MATERIALS AND METHODS

### Biotope loss

The assessment of the status of hypersaline biotopes was possible through different tools: bibliographic data recopilation, in situ prospections and *Artemia* cysts sampling, including some



**Figure 1.** Group centroids of the populations studied for the first two discriminant functions resulting from the discriminant analysis on female morphometric variables. *Grupo de centroides obtenidos para las poblaciones estudiadas a partir de las dos primeras funciones del análisis discriminante aplicado a las variables morfológicas de los especímenes hembras.*



**Figure 2.** Group centroids of the populations studied for the first two discriminant functions resulting from the discriminant analysis on male morphometric variables. *Grupo de centroides obtenidos para las poblaciones estudiadas a partir de las dos primeras funciones del análisis discriminante aplicado a las variables morfométricas de los especímenes machos.*

cyst samples obtained from studies of *Artemia* dispersal by waterbirds (Green *et al.*, 2005).

Many departmental (autonomic) government administrations and cultural foundations in Spain, aware of the importance of salt exploitations as cultural, ethnographic, historical and economic heritage, have developed important inventories of these settlements, reporting on their status of activity. This information was fully available from the environmental departments of Junta de Andalucía (Pérez Hurtado de Mendoza, 2004), Cabildo Insular de Lanzarote in the Canary Islands (Marín and Luengo, 1994), and from Fundació “Sa Nostra” in the Balearic Islands (Frontera, 2005).

Previous engagements arisen from a former INCO-EU project on *Artemia* biodiversity at global scale, together with several international projects (Concerted Actions) among Spain (Instituto de Acuicultura de Torre de la Sal), Portugal (University of Porto), Italy (University of Rome) and Greece (University of Thessalonica) allowed the prospection of Portuguese and Italian salterns during 2004-2005.

Finally, previous and in progress collabora-

tions with the “Estación Biológica de Doñana (CSIC)” and the University of Cadiz in Spain, allowed to verify the presence of formerly known autochthonous *Artemia* populations and/or the lately introduction of the exotic invasive *A. franciscana* (Amat *et al.*, 1995; Green *et al.*, 2005).

The information provided by literature (period 1994-2005) for Spain, and that obtained after the different prospective and sampling campaigns (2004-2005) for Portugal and Italy, was compiled and compared with the information previously available for the region (Amat *et al.*, 2005).

### Invasion extent

The *Artemia* populations studied for the first time and the populations with modified species composition since Amat *et al.*, (2005) are listed in Table 1 (A). These populations were obtained from cyst samples collected in the prospections developed during the period 2004-2006. The populations already cited in Amat *et al.*, (2005), with species composition unchanged, are listed in Table 1 (B).

Part of the information presented in Amat *et*

**Table 1.** List of populations studied for the first time and other populations with updated status since Amat *et al.*, (2005) (A), and those with status unchanged and already cited in this report (B). Geographical coordinates and *Artemia* species and strains are given: AS, *Artemia salina*; AF, *Artemia franciscana*; PD, diploid; PI, triploid; PT, tetraploid parthenogenetic strains. *Lista de las poblaciones estudiadas por primera vez y de aquellas cuya composición ha sido actualizada a partir de Amat et al., (2005) (A), y de las que no han sufrido ningún cambio posterior (B). Se especifican las coordenadas geográficas y las especies o cepas de Artemia halladas: AS, Artemia salina; AF, Artemia franciscana; PD, PI, PT, cepas partenogénicas diploide, triploide y tetraploide.*

Origin	Geographical coordinates	Species or strain	
<b>A. Populations studied for the first time and other populations with updated status (Amat <i>et al.</i>, 2005).</b>			
PORTUGAL			
<i>Algarve District</i>			
Castro Marim (MarVaz Saltern)	37°13'4" N	7°26'16" W	AF
Tavira (Santa Luzia Saltern)	37°6'25" N	7°38'38" W	AF
Olhão (Belamandil Saltern)	37°1'27" N	7°52'3" W	AF
<i>Tagus Estuary</i>			
Alcochete (Marinha Brito Saltern)	38°44'44" N	8°58'37" W	AF
Rio Maior Salterns	39°21'47" N	8°56'33" W	PD
<i>Aveiro District</i>			
Aveiro (Senitra Saltern)	40°38'37" N	8°39'57" W	PD
ITALY			
<i>Apulia Province</i>			
Margherita di Savoia Salterns	41°22'59" N	16°5'20" E	AF, PD
Torrecolimena Saltern	40°18'7" N	17°43'55" E	PD
Cervia Saltern	44°15'3" N	12°20'17" E	PI
<i>Sardinia</i>			
Cagliari (Contivecchi Salterns)	39°13'49" N	9°2'0" E	AS, PD
Cagliari (Molentargius Salterns)	39°13'43" N	9°12'25" E	AS, PD
Sant'Antioco Salterns	39°3'53" N	8°32'27" E	AS
<i>Sicily (Trapani)</i>			
Trapani (Maria Stella Salterns)	37°59'35" N	12°32'8" E	AS
FRANCE			
<i>Languedoc-Roussillon</i>			
La Palme-Sigean Salterns	42°58'30" N	3°1'33" E	PD
<i>Provence</i>			
Salin de Giraud Salterns	43°30'52" N	4°35'56" E	PD
SPAIN			
<i>Cádiz Province</i>			
Puerto de Santa María (La Tapa Saltern)	36°35'52" N	6°13'7" W	AF
Prado del Rey (El Bosque Saltern)	36°47'12" N	5°33'24" W	PD
<i>Tarragona Province</i>			
Ebro River Delta (La Trinidad Saltern)	40°34'58" N	0°40'49" E	AF, AS
<i>Burgos Province</i>			
Poza de la Sal Salterns	42°40'6" N	3°30'0" W	PT
<i>Guadalajara Province</i>			
Imón Salterns	41°9'33" N	2°43'39" W	PT
<i>Huelva Province</i>			
Doñana National Park (San Isidro Salterns)	36°52'43" N	6°21'24" W	AS
<i>Alicante Province</i>			
Santa Pola (Bras del Port Salterns)	38°11'21" N	0°36'21" W	AS, PD
Santa Pola (Bonmatí Salterns)	38°10'5" N	0°37'21" W	AS, PD, PT

Table 1. cont.

Origin	Geographical coordinates	Species or strain	
<b>B. Populations already cited in Amat <i>et al.</i> (2005) with status unchanged.</b>			
<b>PORTUGAL</b>			
<i>Algarve District</i>			
Olhão Salterns	37°1'27" N	7°51'21" W	AF
<i>Sado Estuary</i>			
Bom Fim Salterns	38°24'21" N	8°34'36" W	AF
Rio Frio Salterns	38°24'21" N	8°34'36" W	AF
<i>Aveiro District</i>			
Aveiro (Esmolas Saltern)	40°39'25" N	8°41'26" W	AF
<b>ITALY</b>			
<i>Lazio Province</i>			
Tarquinia Salterns	41°18'34" N	13°20'44" E	AS
<i>Sardinia</i>			
Carloforte Salterns	39°7'59" N	8°18'13" E	AS
<b>FRANCE</b>			
<i>Languedoc-Roussillon</i>			
Aigües Mortes Salterns	43°33'58" N	4°11'28" E	AF, PD
Sète Salterns	43°23'1" N	3°37'59" E	AF
<b>SPAIN</b>			
<i>Cádiz Province</i>			
Sanlúcar (Rocío Saltern)	36°52'39" N	6°19'47" W	AS, PD
San Fernando (El Estanquillo Saltern)	36°25'53" N	6°12'58" W	AF
San Fernando (El Pilar Saltern)	36°29'11" N	6°9'57" W	AF
Puerto Real (Dolores Saltern)	36°30'47" N	6°9'40" W	AF
Puerto Real (San Pascual Saltern)	36°30'47" N	6°9'40" W	AF
<i>Huelva Province</i>			
Odiel River Salterns	37°15'1" N	6°59'26" W	PD, PT
<i>Tarragona Province</i>			
Ebro River Delta (La Trinidad Saltern)	40°34'58" N	0°40'49" E	PT
<i>Alicante Province</i>			
Torre Vieja (La Mata Lagoon)	38°2'8" N	0°42'30" W	AS, PD
<i>Murcia Province</i>			
San Pedro del Pinatar Salterns	37°49'0" N	0°46'0" W	AS
<i>Alava Province</i>			
Añana Salterns	42°48'2" N	2°59'8" W	PT
<i>Albacete Province</i>			
Pétrola Salterns	38°50'0" N	1°33'34" W	PT
<b>MOROCCO</b>			
<i>El Jadida District</i>			
Oualidia (Salines Marocaines)	32°44'50" N	9°1'39" W	AS
U.S.A. ( <i>A. franciscana</i> reference)			
<i>California</i>			
San Francisco Bay	37°30'11" N	122°12'11" W	AF
<i>Utah</i>			
Great Salt Lake	40°57'51" N	112°20'51" W	AF



*al.*, (2005) was inferred from the cyst biometry of the samples that had lost their hatching capability. This species identification method is much less precise than the adult morphology analysis. For this reason, in the present study samples of cysts obtained in the same geographical localities, but in recent collections (2004-2005), as well as those collected in other places, have been used. These samples have been hatched successfully, thus obtaining adult populations after nauplii rearing in standardized conditions. This has allowed its morphometric study through discriminant analysis and, consequently, a complete and precise species identification of all the populations studied. The detailed study of the morphology is the fastest way to ascertain the adscription of a population of *Artemia* to the species to which it belongs. This is due to the similarity of the morphology among the different *Artemia* species, that are considered "sibling" species.

The samples of *Artemia* cysts were cleaned and treated in all cases with standardized methods (Vanhaecke & Sorgeloos, 1980) and included in the cyst bank maintained in the IATS. Batches of nauplii were hatched from these cysts after their incubation in sea water (salinity 35 gL<sup>-1</sup>, temperature 28°C, continuous light, and aeration by air bubbling). These nauplii were grown in a culture medium made of a mixture of the microalgae *Dunaliella salina* and *Tetraselmis suecica*, maintained at salinity 70 gL<sup>-1</sup>, 24 ± 1°C temperature, and 12:12 h light/darkness regime. Complete medium renewal was performed every 2-3 days.

The cultures were maintained in these conditions until 50 % of the females in a batch showed the ovisac developed with their first brood. At this moment a visual identification of the specimens was carried out under binocular microscope. This allowed ascertaining whether the original population was pure or a mixture of several strains. After this previous selection, a multivariate discriminant analysis on the morphometric characters was performed to confirm the systematic adscription of the sample individuals. Samples of 30 females (in parthenogenetic populations as well as in the bisexuals) and 30 males (bisexual populations) were randomly taken, anesthetized, and the morphome-

tric traits of their specimens measured under a binocular microscope equipped with a micrometric eyepiece. The morphological traits and the data processing details are extensively explained in Hontoria & Amat (1992a). The statistic package SPSS for Windows 13.0 (SPSS Inc., Chicago, Illinois, U.S.A.) was used for all the computations. The data were added to the morphological database maintained in the IATS.

### Life tables

Eight *Artemia* populations were chosen for this study, with two representatives for every species or strain (Table 1): San Pedro del Pinatar (Spain) and Salines Marocaines (Morocco) for the bisexual *A. salina*; Margherita di Savoia (Italy) and Ebro River Delta (Spain) for the bisexual invasive *A. franciscana*; Rio Maior (Portugal) and Margherita di Savoia (Italy) for the diploid parthenogenetic, and Imón (Spain) and Ebro River Delta (Spain) for the tetraploid parthenogenetic.

Three to five batches of 30 nauplii from every population were isolated into 50 mL container aliquots. Culture medium conditions described above were provided to these nauplii. They were monitored every 2 to 3 days to renew completely the medium and to evaluate the survival. When young females showed ovulation, previously to the first brood, they were isolated, individually (parthenogenetic) or with a male (bisexuals), in 50 mL containers under the same culture conditions. Monitoring of the life span and reproduction, and culture medium renewal, were performed every two-three days. Dead males in bisexual matings were replaced from mass culture containers kept under the same conditions. Life table parameters monitored were the length of prereproductive (time to attain the first brood), the reproductive (time of active reproduction from the first to the last broods), and the postreproductive (time elapsed between the last brood and death) periods. The mean time elapsed between broods and the total life span, in days, were followed. In addition, the total number of broods per female, and the total offspring per female, per brood and per day, were also monitored. Finally, the type of

reproduction: ovoviviparous/encysted, and the quality of the ovoviviparous offspring (living or dead nauplii together with abortive embryos or unfertilized eggs) were assessed.

### Statistics

The means of the variables obtained in the life tables for the different *Artemia* populations have been compared through the Brown-Forsythe's test in search of differences among them, followed by the Games-Howell's test for multiple mean comparisons. Both tests are robust in cases of variance heteroscedasticity, which have been very frequent in our study due to the high dispersion that these traits show. In fact, only the mean time elapsed between broods has proved to present homogeneous variances. In addition, the variables quantified as percentage lack of the necessary normality and, consequently, it is not advisable the use of the above mentioned statistical treatment. Instead, non parametric statistics (Kruskal-Wallis' tests) have been employed to assert mean differences in these variables. Statistical analyses were performed with the SPSS 14.0 for Windows package (SPSS Inc., Chicago, IL, USA).

## RESULTS

### Biotope loss

The loss of hypersaline biotopes, especially the solar salterns adequate for the development of brine shrimp populations, attains 74 % in Spain, 63 % in Italy and about 55 % in Portugal (Table 2).

### Invasion extent

The widespread presence of *A. franciscana* in the Western Mediterranean region is confirmed (Table 1) after its populations identification by adult biometry (Figs. 1 and 2). This is specially remarkable almost in all the saltworks from Portugal. However, two salterns isolated from the important saltworks areas have been also visited: the Rio Maior saltern (39°21' N-8°56' W), an inland saline spring close to the city of Santarem, and the Senitra saltern (40°38' N-8°39' W),

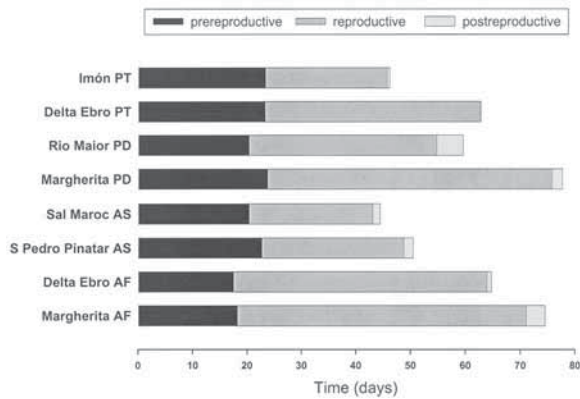
**Table 2.** Approximate distribution of habitat loss, mainly solar salterns, in the Western Mediterranean region during the last 20 years, particularized for countries and regions. Data given as percentages. *Distribución aproximada de los hábitats perdidos, principalmente salinas solares, en la región del Mediterráneo Occidental durante los últimos 20 años, particularizada por países y regiones. Los datos se dan como porcentajes.*

<b>SPAIN</b>	<b>74</b>
Catalonia	50
Valencia	45
Murcia	65
Aragon	85
Navarre	100
Castile-La Mancha	80
Andalusia*	75
Balearic Islands	75
Canary Islands	85
<b>ITALY</b>	<b>63</b>
Veneto	100
Apulia	30
Lazio	100
Sardinia	60
Sicily	25
<b>PORTUGAL</b>	<b>55</b>
Algarve	25
Sado Estuary	80
Tagus Estuary	75
Mondego Estuary	20
Aveiro	50
*67 % continental, 82 % coastal	

close to the saltworks complex of the Aveiro district. Both salterns, with small extension and traditional exploitation, show the presence of diploid parthenogenetic strains belonging to the autochthonous populations of *Artemia*. These are the two only native populations found in Portugal compared to the extensive presence of *A. franciscana*, from the South (Algarve) to the North (Aveiro).

In Italy the appearance of a population of the exotic form *A. franciscana* in the Margherita di Savoia salterns (41°22' N-16°5' E) on the Adriatic coast, in the province of Apulia (Mura *et al.*, 2004, Mura *et al.*, 2005a) has been recently verified. The first evidences of its introduction come from the study of a cyst sample collected



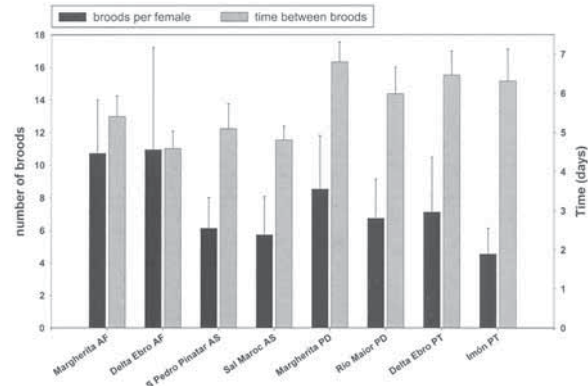


**Figure 3.** Time elapsed during prereproductive, reproductive and postreproductive periods, and total life span, in days, obtained from the life tables studied for populations (8) from different species and strains (4) under experimental static conditions. *Duración de los períodos prereproductivo, reproductivo y postreproductivo, y de la vida total, en días, obtenida del estudio de tablas de vida para poblaciones (8) de diferentes especies y cepas (4) bajo condiciones de experimentación estáticas.*

in February 2004. The population obtained in the laboratory from these cysts confirmed that the higher fraction of *Artemia* was autochthonous diploid parthenogenetic individuals, with a scarce presence of *A. franciscana* (1.20 % of individuals). The samples obtained during the next summer season show the dominant presence of the invasive species. Other worth mentioning localities are the Cervia salterns (44°15' N-12°20' E) in the Adriatic littoral, and Torrecolimena salterns (40°18' N-17°43' E), in the Tarentum Gulf (Ionic Sea) (Mura *et al.*, 2005b), housing exclusively autochthonous parthenogenetic populations.

In the important Sardinian saltern complexes of Cagliari (Molentargius and Contivecchi) and in Sant'Antioco, as well as in the Trapani-Paceco saltern complex (Maria Estela saltern) from Sicily, native *A. salina* (bisexual) and diploid parthenogenetic populations have been exclusively found.

In France *A. franciscana* is present in the saltern complexes from Sète-Aigües Mortes (43°3' N-4°11' E) in the Languedoc, in the large salterns in Salin de Giraud (Etang de Vaccarés, 43°30' N-4°3' E) and those in Provence. However, different diploid parthenogenetic populations have been located in hypersaline ponds in La Palme-Sigean (42°58' N-3°1' E).

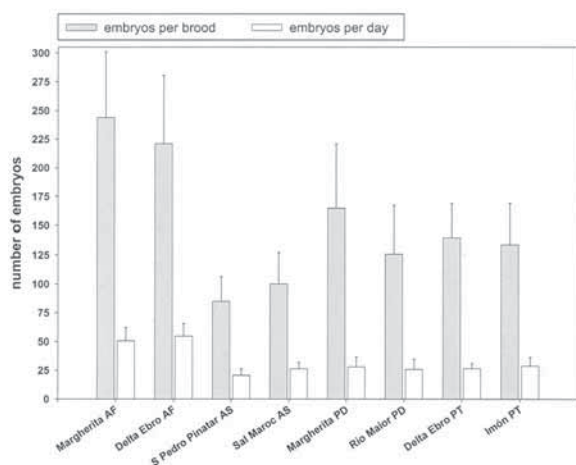


**Figure 4.** Total number of broods per female, and time elapsed between broods, obtained from the life tables studied for populations (8) from different species and strains (4) under experimental static conditions. *Número total de puestas emitidas por hembra, y duración del período transcurrido entre puestas, obtenidos del estudio de tablas de vida para poblaciones (8) de diferentes especies y cepas (4) bajo condiciones de experimentación estáticas.*

In Spain, different sampling campaigns have allowed to add to the population inventory new autochthonous populations as the diploid parthenogenetic from El Bosque in the province of Cádiz (36°47' N-5°33' W) and the parthenogenetic tetraploid from Poza de la Sal in the province of Burgos (42°40' N-3°30' W). In addition, these recent prospectations have evidenced the presence of new *A. franciscana* introductions as in the River Ebro Delta salterns (Alfaques Bay, province of Tarragona) (40°34' N-0°40' E). Other recently invaded localities are La Tapa saltern (36°35' N-6°13' W) in Puerto de Santa María (Cádiz), and Fuente de Piedra lagoon (36°45' N-5°31' W) in the province of Malaga. These data support the idea that most *Artemia* habitats of this Atlantic area in the South of Spain and Portugal are already colonized by the exotic invasive species.

### Life tables

The observation of temporal parameters obtained from life tables, as shown in figure 3, allow to confirm that *A. franciscana* females develop a prereproductive period markedly shorter (18 days) than the females from all the other autochthonous populations (20 to 30 days). Brown-

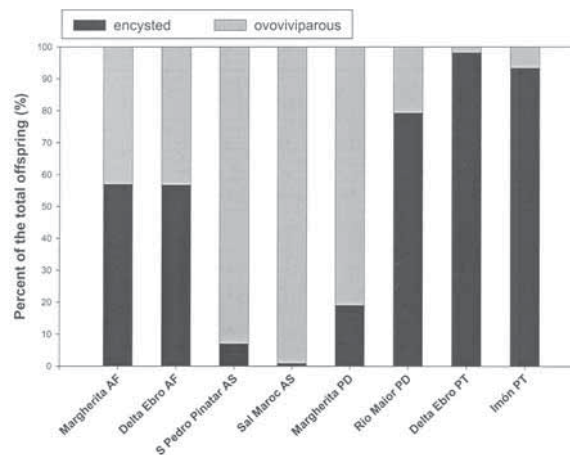


**Figure 5.** Fecundity in terms of embryos per brood and per day, per female, obtained from the life tables studied for populations (8) from different species and strains (4) under experimental static conditions. *Fecundidad en términos de número de embriones obtenidos por puesta, por día y por hembra, obtenida del estudio de tablas de vida para poblaciones (8) de diferentes especies y cepas (4) bajo condiciones de experimentación estáticas.*

Forsythe's plus Games-Howell's test show significant differences ( $p \leq 0.05$ ) between *A. franciscana* and all the other species for this trait.

Females from both bisexual species (*A. franciscana* and *A. salina*) show a similar time period elapsed between successive broods, while this period is always longer for parthenogenetic females. The reproductive period and total life span are very similar for both *A. franciscana* and most of the diploid parthenogenetic females, as in the case of the parthenogenetic strain from Margherita di Savoia. However, this is not true for the Rio Maior strain. The postreproductive period seems to be negligible in all cases and presumably does not contribute to the assessment of the biological fitness. Total life span is markedly shorter for the females of the bisexual autochthonous *A. salina* populations, attaining only 40 to 50 days.

The quantitative traits for reproduction show markedly higher values in *A. franciscana*, especially in important outputs as the total number of broods per female (Fig. 4) and total offspring per brood and per day (Fig. 5). The exotic *A. franciscana* females show the largest va-

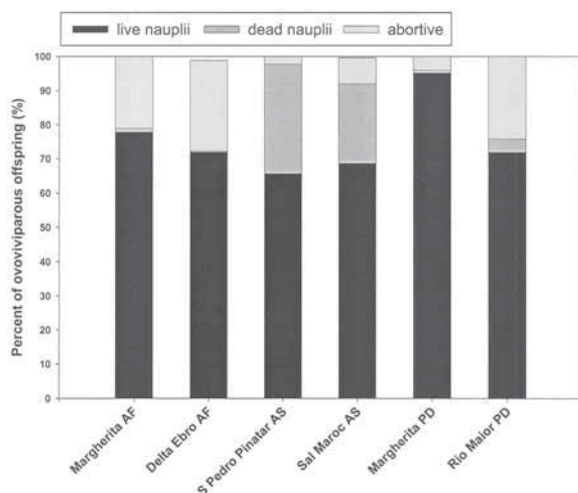


**Figure 6.** Qualitative reproduction in terms of percentage of the total ovoviviparous vs. encysted offspring, obtained from the life tables studied for populations (8) from different species and strains (4) under experimental static conditions. *Calidad reproductiva en términos de porcentaje de puestas ovovivíparas versus ovíparas (enquistadas) sobre el total de puestas, obtenida del estudio de tablas de vida para poblaciones (8) de diferentes especies y cepas (4) bajo condiciones de experimentación estáticas.*

lues for these reproductive outputs, which usually duplicate those of the autochthonous strains. Nevertheless, among the autochthonous strains, the reproductive outputs are always higher for the parthenogens, compared to those of the bisexual *A. salina*. Brown-Forsythe's plus Games-Howell's tests show significant differences ( $p \leq 0.05$ ) for time elapsed between broods and total offspring produced per day between *A. franciscana* and all the other species for both traits, being the former the shorter and the latter the bigger for the exotic species among the experimental populations, respectively.

A marked oviparism (encysted offspring) was found in the parthenogenetic strains, which is nearly exclusive in tetraploid strains (Barata *et al.*, 1995). However, the Margherita di Savoia diploid parthenogenetic females are an exception in this case. In opposition to parthenogens, the bisexual *A. salina* females display scarce oviparism, while *A. franciscana* females show a substantial balance between oviparism (57% Fig. 6) and ovoviviparism.

Some remarks dealing with the quality of ovoviviparous offspring, usually not considered



**Figure 7.** Differences in qualitative ovoviviparous offspring in diploid (bisexual and parthenogenetic) species and strains, with expression of relative percentages of live and dead nauplii, and abortive embryos, obtained from the life tables studied for populations (8) from different species and strains (4) under experimental static conditions. *Diferencias cualitativas halladas en la descendencia ovovivípara de especies y cepas diploides (bisexuales y partenogénicas), especificando los porcentajes relativos de nauplios vivos, muertos y de embriones abortivos, obtenidas del estudio de tablas de vida para poblaciones (8) de diferentes especies y cepas (4) bajo condiciones de experimentación estáticas.*

in the literature available are worth to mention. Ovoviviparous broods typically comprise living and dead nauplii, together with abortive embryos showing arrested development. Sometimes they are remnant ovocytes not properly fertilized by males in bisexual species. The quality of these ovoviviparous outputs should be considered when estimating the reproductive and invasive success. In this work it has been possible to discern the presence of 20 % to 30 % of the ovoviviparous offspring as abortive in the two *A. franciscana* populations studied. While similar percentages of dead nauplii are present in the autochthonous bisexual *A. salina*, the diploid parthenogenetic strain from Margherita di Savoia displays a high level of ovoviviparous quality attaining the 95 % of the ovoviviparous output as living nauplii (Fig. 7). An exception to this is the case of the Rio Maior population, which shows a living nauplii proportion of about 75 % of the ovoviviparous progeny, together with a marked tendency for encysted offspring.

## DISCUSSION

We report evidences of the dramatic loss of hypersaline habitats in the Mediterranean basin. In Spain one of the most accurate and strict inventories was performed in the Andalusian Region, where 36 solar salterns are today in exploitation out of the 185 accounted for two or three decades ago. It is also surprising the fact that the remaining are mostly inland (33 %) with fewer littoral salterns remaining active (18 %). The perdurability and fate of these small and traditionally managed inland exploitations is threatened by scarce rentability and returns. The littoral small exploitations are affected by the same threats, but also by the pressure to switch to more productive activities like intensive fish culture or urban projects for tourism and leisure.

Something similar is happening in Portugal and Italy. Portuguese salterns in the estuary of the Sado River switched to rice fields, while fish culture is developed massively in the estuary of the Tagos River. Some peninsular salterns in Italy were abandoned after recent floods (Comacchio in the Venetian region, Tarquinia in the Lazio), while most Eastern Sicilian salterns switched to agriculture (Pachino-Marzamemi).

The previous report by Amat *et al.*, (2005) stated the widespread presence of *A. franciscana* in the hypersaline ecosystems from Portugal. This presence could have started in the decade of 1980 (Hontoria *et al.*, 1987), when it was observed for the first time in the southern salterns, in the district of Algarve. At that time, *A. franciscana* was not present in the northern Portuguese regions, in the district of Aveiro (Vieira & Amat, 1985), and it has been likely progressing from South to North.

The time and place of the original introduction of the exotic species in Portugal is completely unknown, and could have been a human decision (aquaculture), but from that moment the presumable dispersion via water birds begun along the East Atlantic flyway, West of Gibraltar (Green *et al.*, 2005). This hypothesis could be confirmed after verifying the taxonomic adscription of the bisexual *Artemia* populations that presently thrive in La Guerande salterns (South of Bretagne,

France) (Joseph Baudet, personal communication, 2005). The presence of parthenogenetic tetraploid brine shrimp populations in these localities (Amat, 1983) has been known from the end of the 1970's decade. If present populations are bisexual, it is hard to believe that they belong to the Mediterranean bisexual *A. salina*, unsuitable for these northern latitudes.

A similar dispersive phenomenon related to the existence of the West Mediterranean flyway, East of Gibraltar (Green *et al.*, 2005), favoring an *Artemia* dispersion via water birds is still uncertain. Nevertheless, the massive presence of *A. franciscana* in most of the active salterns in Cádiz Bay is well known (Amat *et al.*, 2005). More pieces of evidence pointing towards this hypothesis come from the recent (2005) findings of *A. franciscana* in the exploited La Tapa salterns (Puerto de Santa María, Cádiz) and in the Fuente de Piedra (Málaga) inland hypersaline lagoon, the most important nesting area for flamingos in the Iberian Peninsula, and where authors are not aware of any introduction by humans.

The presence of *A. franciscana* in the Ebro Delta salterns (Alfaques Bay, Tarragona) (Figs. 1 and 2), as well as in the French salterns in Sète and Aigües Mortes (Languedoc-Roussillon) (Thiéry & Robert, 1992; Amat *et al.*, 2005) could support the suspected role of water birds as dispersors of *A. franciscana* through their West Mediterranean flyway, probably also reaching the Italian salterns in Margherita di Savoia (Apulia) in the Adriatic Sea (Mura *et al.*, 2004). In any case, the massive inoculation of the exotic species in important industrial salterns in the Mediterranean basin must not to be dismissed.

These hypothetical inoculations (anthropic pressure), together with the presence of water birds in marine solar salterns as breeding and wintering sites, increase the probabilities for dispersion of the exotic invasive *A. franciscana*. However, the presence of autochthonous brine shrimp species and strains in small inland salterns, and in isolated sea shore salterns, like Rio Maior (Santarem) and Senitra (Aveiro) in Portugal; El Bosque (Cádiz), Añana (Alava), Poza de la Sal (Burgos) in Spain, or Torrecolimena (Apulia), Molentargius (Sardinia),

Trapani (Sicily) in Italy, offer some hope for the maintenance of *Artemia* biodiversity in the Western Mediterranean. However, these small ecosystems are also threatened of abandonment by lack of economical interest, and so is the *Artemia* biodiversity.

Some Mediterranean countries are developing interesting projects to repair solar salt exploitations, old sites witnessing important cultural, ethnographic and historical roots. This is the case for Añana (Alava), Poza de la Sal (Burgos), Imón (Guadalajara), in Spain, similarly to the Ettore-Infersa salt complex (Marsala-Trapani) in Sicily (Italy), or Rio Maior in Portugal. In all these small inland and marine salterns it is possible to find the suitable conditions for the persistence of the autochthonous *Artemia* species and strains (*A. salina* and parthenogenetic forms). These sites are out of the main water bird flyways, although they are not free of the anthropic intervention in terms of exotic species inoculations or an accidental introduction by the small number of birds that visit the area. This has already happened in Gerri de la Sal (Baix Pallars, Lleida) salterns, where the introduction of *A. franciscana* in the last decades of the twentieth century eliminated the autochthonous diploid parthenogenetic strain.

Environmental factors like salinity, temperature, food or oxygen availability, or factors related to the population dynamics, like the different species cooccurrence, crowding, type of reproduction, rule the response of different populations in terms of biological fitness and life span (Browne *et al.*, 1984, 1988, 1991; Barata *et al.*, 1995, 1996a,b). Among these traits, the length of prereproductive and reproductive periods has the most important effect on organismal fitness (Allan, 1976).

The biological fitness assessed in this research was developed in static conditions, according to criteria supported by Browne (1980) and Browne & Halanych (1989), enabling to build up comparative and competing results for bisexual *A. salina* and *A. franciscana* species, as well as for parthenogenetic strains. Several authors have evaluated the combined effects of salinity and temperature on biological fitness for diverse populations of these strains and species (Browne *et al.*, 1988, Browne & Wanigasekera, 2000; Barata *et al.*, 1996b). In all cases it has been



stated that the Mediterranean bisexual *A. salina* is the less tolerant to high temperatures. The responses in terms of biological fitness (longer life span) associated to higher temperatures are in favor of the exotic species *A. franciscana*, even with a broader tolerance than that of the parthenogenetic strains.

The autochthonous *Artemia* species and parthenogenetic strains present adaptive and tolerance mechanisms to well defined ranges of environmental changes. The bisexual *A. salina* prefers low temperatures but does not perform properly at salinities lower than  $60 \text{ g L}^{-1}$  (Amat, 1983; Barata *et al.*, 1996b; Browne & Wani-gasekera, 2000). In the Mediterranean basin the bisexual and the diploid parthenogenetic populations can coexist in the same saltern. Usually there are conditions favorable to *A. salina* early in the year at lower temperatures and salinities, with a later seasonal dominance for parthenogens at higher temperatures and salinities. Barata *et al.*, (1996a) reported the competitive displacement of *A. salina* by parthenogenetic strains at  $24^\circ\text{C}$ , while the former performs better than the parthenogenetic at  $15\text{-}20^\circ\text{C}$ .

Our results are in agreement with the above generalizations. The exotic *A. franciscana* populations introduced in Margherita di Savoia and River Ebro Delta display the longest reproductive periods studied. The diploid parthenogenetic strain from Margherita di Savoia shows a similar reproductive period length (50 days). In addition, the exotic species shows shorter prereproductive and between brood periods. When autochthonous strains release their first brood, the exotic species has already produced two offspring batches. If these are ovoviviparous nauplii, their presence trigger the competitive superiority developed by *A. franciscana* in terms of total offspring per batch, per day and per female. It is possible to observe (Figs. 3 to 7) the regularity in offspring production developed by the *A. franciscana* populations studied, with a twofold offspring output per female and per day in comparison with the autochthonous strains. The significant differences found between both *A. franciscana* populations and all the other species populations for those three basic fitness traits (prereproductive and between brood

periods, together with offspring output per female and per day) support the competitive superiority developed by the exotic invasive species, what has been observed recently in competitive studies in open air microcosm in progress.

According to Browne *et al.*, (1984) and Lenz & Browne (1991) the greatest differences in reproductive output, in terms of fecundity and offspring quality, are found between the bisexual species *A. franciscana* from the American continent and the Mediterranean *A. salina*. Sometimes these differences are not so clear between the bisexual American species and the Old World diploid parthenogenetic strains, although *A. franciscana* females display the greatest fecundity of all, as stated in this work.

There is a powerful argument that can be presented to explain the failure of the putative superiority in some bisexual species on the basis of their reproductive output. Mediterranean *A. salina* is a clear example. Bisexuals must pay for the meiosis cost. Females need to be fertilized by sexual males. Bisexual offspring production needs the presence of the two sexes, which is irrelevant for parthenogens.

The tetraploid parthenogenetic females display low fecundity, although their bigger size and biomass could be linked to higher fecundities (Zhang & King, 1993). This lower offspring production is also more remarkable due to the dominance of oviparism (encysted offspring), a more costly reproductive mode than ovoviviparism, because 22 % of the dry mass of a cyst batch is utilized for encapsulation (Clegg, 1962; Von Hentig, 1971, in Barata *et al.*, 1995). This is true even when there is some level of ovoviviparism, producing embryos and nauplii of bigger size than the other diploid species or strains, due to the polyploidy. Polyploid *Artemia* strains are usually spread in continental, as well as in high latitudinal, hypersaline environments, although they have been recently found in solar salterns in Northern Africa (Morocco, Algeria, and Tunisia). This distribution and type of reproduction, bound to some quality of "robustness" associated to polyploids and colonizing strains, is also linked to an adaptation to flourish during the short length of the inhabitable period of

their inland uncertain environments (Browne *et al.*, 1984, 1988; Barata *et al.*, 1995).

A few decades ago the exotic species *A. franciscana* appeared into the Mediterranean landscape. In addition to better performances in biological fitness, the exotic species displays wider eurythermal and euryhaline capabilities (Browne & Wanigasekera, 2000). These are advantages characterizing a “super species” spread unrestrictedly along the American continent. The only exception is the Patagonian region in the American South Cone, where *A. persimilis* is still enduring a hypothetical invasion by *A. franciscana* from the North (Amat *et al.*, 2004). The super-species, endowed with high levels of phenotypic plasticity (Abatzopoulos *et al.*, 2002), becomes an invasive species in Asia, Africa and Europe, where outcompetes the autochthonous species, which are laid aside and eliminated in all cases. This situation agrees completely with laboratory experimental results, where diverse organisms, different from the brine shrimp, display practical exclusion among congeneric species into 10 to 100 generations (Miller, 1967), but in the case of *Artemia*, according to Lenz & Browne (1991), and our own unpublished results, exclusion may be attained after only 2 to 3 generations.

The displacement or elimination of most autochthonous populations in our latitudes is evident in Portugal and France, and runs progressively in continental Spain and Italy. This is in progress not only in the Western Mediterranean, but it is being reported also in the Middle and Far East, i.e. in Iraq, India, Pakistan, China, countries where autochthonous species existed before. Massive introductions are only conceivable through antropic intervention, and probably this is the only way to explain its fast progression in sites like Margherita di Savoia (Italy). In a similar way it is possible to explain the present situation in the salterns of the Hebei province in China (Tangu, Luanan, Dagang, from own unpublished results) where, in the last five to ten years, autochthonous bisexual *A. sinica* and diploid parthenogenetic populations have been practically eradicated, changing to a nearly exclusive predominance of the exotic invasive *A. franciscana*.

Once these sites of massive introduction have been established, a broad dispersion East and Westwards in the Old World, at short and middle term, is warranted through the cyst exchange performed among salt exploitations and aquacultural development. Together with the unavoidable dispersion via migrating water birds, a long term important loss of *Artemia* biodiversity into natural environments is conceivable. Only exceptionally isolated hypersaline ecosystems could harbour autochthonous strains, not completely free from threatening introductions, similarly to what is happening in the Western Mediterranean.

In the present situation it is difficult to anticipate any particular measure to prevent or eradicate the exotic brine shrimp species, similarly to what usually is applied to invaders. The oviparism mode of reproduction warrants the persistence of *Artemia* in any invaded or colonized ecosystem. Otherwise, a possible way to attain a circumstantial suppression of the living populations of an invader species could be some kind of “biological fight” avoiding any damage to the environment itself. This biological fight could consist, for example, on massive introductions of the invader species adequately sterilized (ionic irradiations, xenobiotics), together with massive reinoculations of the autochthonous species. In any case, further scientific research on these proposals is necessary, provided their understanding and support by social, political and economical authorities.

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