

The American brine shrimp as an exotic invasive species in the western Mediterranean

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Abstract

The hypersaline environments and salterns present in the western Mediterranean region (including Italy, southern France, the Iberian Peninsula and Morocco) contain autochthonous forms of the brine shrimp *Artemia*, with parthenogenetic diploid and tetraploid strains coexisting with the bisexual species *A. salina*. Introduced populations of the American brine shrimp *A. franciscana* have also been recorded in these Mediterranean environments since the 1980s. Based on brine shrimp cyst samples collected in these countries from 1980 until 2002, we were able to establish the present distribution of autochthonous brine shrimps and of *A. franciscana*, which is shown to be an expanding invasive species. The results obtained show that *A. franciscana* is now the dominant *Artemia* species in Portuguese salterns, along the French Mediterranean coast and in Cadiz bay (Spain). Co-occurrence of autochthonous (parthenogenetic) and American brine shrimp populations was observed in Morocco (Mar Chica) and France (Aigues Mortes), whereas *A. franciscana* was not found in Italian cyst samples. The results suggest these exotic *A. franciscana* populations originate as intentional or non-intentional inoculations through aquacultural (hatchery effluents) or pet market activities, and suggest that the native species can be rapidly replaced by the exotic species.

Introduction

The brine shrimp *Artemia* (Branchiopoda, Anostraca) is perhaps the most conspicuous inhabitant of hypersaline lakes and lagoons and solar saltern ponds, coastal and inland, over the world, where simple trophic structures and low species diversity are present (Lenz and Browne 1991).

The genus *Artemia* comprises a group of bisexual and parthenogenetic species, which probably diverged five to six million years ago from an ancestral form living in the Mediterranean area (Abreu-Grobois and Beardmore 1982; Badaracco

et al. 1987). The string of shallow briny lakes into which the Mediterranean sea had converted (Hsü et al. 1977) created opportunities for colonization, extinction and recolonization cycles with different degrees of reproductive isolation, while the appearance of a parthenogenetic mode of reproduction, together with polyploidy, may have facilitated dispersion. Thus, the Mediterranean has been proposed as the centre of radiation for *Artemia*, based on changes in reproduction modes, bisexuality and parthenogenesis on the one hand, together with diploidy and polyploidy on the other (Gajardo et al. 2002).

On the basis of criteria from morphometry and laboratory reproductive isolation, and subsequently through karyology, allozyme divergence and new molecular (DNA) markers, seven bisexual species and two or three parthenogenetic forms are currently recognized in the genus *Artemia*. They all look rather similar in body shape, but show morphological traits that enable morphometric differentiation when they are cultured under standard laboratory conditions (Hontoria and Amat 1992a, b).

The bisexual *A. persimilis* (Piccinelli and Prodocimi 1968) (Argentina and Chile) and *A. franciscana* (Kellogg 1906) (North, Central and South America) are endemic to the New World. The bisexual *A. salina* Leach 1819 (Mediterranean area and Africa), *A. urmiana* (Günther 1890) from Iran, *A. sinica* (Cai 1989) from P.R. China, and *A. tibetiana* (Abatzopoulos et al. 1998, 2002) from Tibet, with *Artemia* sp. from Kazakhstan (Pilla and Beardmore 1994) are endemic to the Old World. Recently, the American species *A. franciscana* has been introduced in the Old World, especially in the Far East and in the Mediterranean area, as explained in this study.

The parthenogenetic forms, with different degrees of ploidy, are present in the Old World, i.e. Eurasia and Africa, and were introduced in Australia. Although these forms are listed taxonomically with the binomen *Artemia parthenogenetica*, the wide diversity found among different asexual populations, especially in terms of ploidy, suggests that their grouping under a single species may be misleading (Browne et al. 1991; Gajardo et al. 2002). The distribution of *Artemia* populations in the western Old World, including Italy, south of France and the Iberian Peninsula (Spain and Portugal), together with the north of Africa, is especially interesting owing to the presence and distribution of the Mediterranean bisexual *A. salina* and, at least, two different parthenogenetic forms, diploid and tetraploid (Artom 1906; Stella 1933; Gilchrist 1960; Stefani 1960; Amat 1983a, b; Vieira and Amat 1985; Vanhaecke et al. 1987; Amat et al. 1995; Tryanaphyllidis et al. 1997a, b).

This region also shows the unfortunate event of the presence of the American species *Artemia franciscana* (Narciso 1989; Hontoria et al. 1987; Amat et al. 1995). This paper aims to review the current distribution of *A. franciscana* populations

in the western Mediterranean region and their likely origins. We also compare the biometry of both introduced and autochthonous populations, and consider the impact of the exotic species on the native ones.

Materials and methods

This research was carried out using a large collection of *Artemia* cyst samples in the Instituto de Acuicultura de Torre de la Sal (CSIC), supported by the samples available from the *Artemia* Reference Center (University of Ghent, Belgium), and a database of morphometric characterizations of adult specimens from different *Artemia* populations and species. This database used a 'morphometric standard' describing the populations obtained after hatching these cysts in the laboratory and their culture under standard conditions (Hontoria and Amat 1992a).

The cyst collection contained about 130 cyst samples from Western Europe (Spain, Portugal and Italy) collected since 1980. During 2001 and 2002, new cyst samples were taken from the southwest of Spain and Portugal (by the authors), and the southeast of France (provided by Dr Thomas Lenormand, Université de Montpellier, France), the European regions where the invasive presence of *A. franciscana* was first reported (Narciso 1989; Thiery and Robert 1992). We also analysed cysts of asexual *Artemia* that we extracted from the pellets of Redshank *Tringa totanus* that were collected from Cadiz Bay in July and August 2002 (A.J. Green, M.I. Sánchez, F. Amat, J. Figuerola, F. Hontoria, O. Ruiz, F. Hortas, unpublished manuscript).

Cysts from Italy (Dr Graziella Mura, University of Rome) and from Portugal (Dr M^a Elena Vilela, Instituto de Investigação das Pescas e do Mar, Lisbon) were preserved in plastic bags under vacuum. Other cysts samples were processed according to standardized methods (Vanhaecke and Sorgeloos 1980) and stored at 4 °C in sealed plastic bags.

Biometry of adults

The nauplii obtained by cyst hatching were made to grow up in 5 l plastic containers, with 70 g l⁻¹ filtered brine (seawater plus crude sea salt), and

put on a mixed diet of *Dunaliella salina* and *Tetraselmis suecica*. The temperature was maintained at 24 ± 1 °C, under mild aeration at a 12D : 12L photoperiod. The medium was monitored and renewed every 2 days. Once 50% of the females attained full ovisac development and the first ovoviviparous offspring was observed, random samples of 30 females (parthenogenetic and bisexual strains) and 30 males (bisexual strains) were removed from the culture, anaesthetized and measured under a dissecting microscope. The following morphological parameters were quantified in each specimen: total length, abdominal length, width of third abdominal segment, width of the ovisac in females and width of the genital segment in males, length of furca, number of setae inserted on each branch of the furca, width of head, maximal diameter and distance between compound eyes, length of first antenna and the ratio of abdominal length \times 100/total length. The biometrical analysis of these data was performed via multivariate discriminant analysis (Hontoria and Amat 1992a) using the statistical package SPSS for Windows version 11.0 (SPSS Inc., Chicago, Illinois, USA), and the results were integrated in the morphological data base developed at the Instituto de Acuicultura de Torre de la Sal (Amat et al. 1995).

Biometry of cysts

When cysts from old or mishandled samples did not hatch, it was impossible to obtain living nauplii; therefore, to grow up laboratory populations to adulthood. In this case, the population specific adscription was obtained through the biometric study of cysts, which provides a fitting alternative for this purpose (Vanhaecke and Sorgeloos 1980; Hontoria 1990).

Samples providing sufficient quantities of cysts allowed cyst diameter analysis with an electronic Coulter Counter[®] counter-sizer (Vanhaecke and Sorgeloos 1980; Hontoria 1990). The other cyst samples were previously hydrated with 20 g l^{-1} filtered seawater, at 28 °C with continuous illumination and aeration, and measured under a dissecting microscope to the nearest μm . Cysts were hatched under standard conditions: 35 g l^{-1} filtered sea water, at 28 °C, with continuous illumination and aeration.

Several cyst samples did not hatch after the first attempt, and according to the amount of cysts available, they were submitted to two or three hydration and dehydration (24 h oven dessication under 39 °C) cycles, and/or to a H_2O_2 treatment, processes that terminate diapause of *Artemia* cysts (Lavens and Sorgeloos 1987; VanStappen et al. 1998), before a final attempt to hatch them in order to obtain living nauplii.

Results

Biometry of cysts and adults

The information obtained from cyst samples from Portugal is shown in Table 1. Only those samples collected since 1990 hatched. Laboratory populations showed the exclusive presence of *A. franciscana*, based on the morphometric study of adult specimens (Figure 1). However, the information from cyst biometry allows inferring the presence of *A. franciscana* in the Algarve from the beginning of the 1980s. In the Sado estuary area, the situation is similar, but Olhos and Cachopos salterns may still have held *A. parthenogenetica* populations in the 1980s according to the cyst diameter that exceeded $260 \mu\text{m}$ (Hontoria 1990). In the Tejo estuary, it is possible to infer the presence of *A. franciscana* in Alcochete and Boavista salterns in the 1980s, but autochthonous parthenogenetic (diploid and tetraploid) populations (cyst diameter between 260 and $280 \mu\text{m}$) were dominant at that time. Finally, in the Esmolas salterns, from the district of Aveiro, the presence of *A. parthenogenetica* was stated by Vieira (1990), but samples collected in 1991 showed the exclusive presence of *A. franciscana*.

More recent cyst samples from Huelva and Cadiz provinces in Spain, from Mar Chica (Nador, Morocco) and from the South of France hatched successfully. The populations obtained from these cysts verified the presence of the autochthonous bisexual (*A. salina*) and the parthenogenetic diploid and tetraploid strains, together with the exotic *A. franciscana* (Table 2). The Westernmost Spanish populations (Odiel in Huelva and N.S. del Rocío in Sanlúcar de Barrameda, Cadiz) showed the exclusive presence of autochthonous populations (*A. salina* and/or *A. parthenogenetica*) in variable ratios, whereas the Moroccan

Table 1. *Artemia* cyst samples available from Portugal, mean diameter of cysts and taxonomical identification.

Locality	Sampling date	Cyst diameter (μm)			Observations
		Micro	C.C.	C.C.(*)	
<i>Algarve Province</i>					
San Francisco salterns	1980–1981	243	236	238	NH
Marina Bias salterns	1987	246	248	–	NH
Olhao salterns	1985	247	–	259	NH
Tavira salterns	1985	240	–	–	NH
Olhao salterns	2002	253	–	–	<i>A. franciscana</i>
Faro. Ludo salterns	2002	245	–	–	<i>A. franciscana</i>
Castro Marim salterns	2002	250	–	–	<i>A. franciscana</i>
<i>Sado Estuary</i>					
Batalha salterns	1986	236	–	–	NH
Sado salterns	1987	248	–	–	NH
Olhos salterns	1986	262	–	–	NH
Cachopos salterns	1987	276	–	–	NH
Rio Frio salterns	1993	228	–	–	<i>A. franciscana</i>
Bonfim salterns	1996	224	–	–	<i>A. franciscana</i>
<i>Tejo Estuary</i>					
Alcochete salterns	1988	259	256	266	NH
Boavista salterns	1987	258	260	–	NH
Marina Nova salterns	1987	264	273	–	NH
Marina Velha salterns	1987	–	276	–	NH
Providencia salterns	1987	–	276	–	NH
<i>Aveiro District</i>					
Esmolas salterns	1985	266 (**)	–	263	<i>A. parthenog.</i> (d)
Esmolas salterns	1991	249	–	–	<i>A. franciscana</i>
Esmolas salterns	1993	248	–	–	<i>A. franciscana</i>

Micro.: cyst diameter measured with micrometer eyepiece. C.C.: cyst diameter measured with Coulter Counter. C.C.(*): idem (Hontoria 1990); (**) Vieira 1990; NH = No hatching cysts. (d): *Artemia parthenogenetica* diploid.

population showed a mixture of the autochthonous diploid parthenogenetic and the American species. However, the salterns located in Cadiz bay showed the exclusive presence of *A. franciscana*. French samples corresponded mostly to *A. franciscana* populations, verified through specimens obtained under culture or through those provided as preserved samples. In Aigues Mortes, low proportions of the autochthonous parthenogenetic population were recorded.

Cyst samples available from Italy and the information obtained from them are shown in Table 3. Some of these samples, collected before 1987, did not hatch. Laboratory populations obtained from viable cysts showed the presence of the autochthonous populations, i.e., the bisexual *A. salina* and both parthenogenetic strains, diploid and tetraploid. According to data reported previously by Hontoria (1990), the cysts from the Comacchio salterns correspond to a parthenogenetic tetraploid strain because of their

big size, while the Sicilian sample from Isola Longa (Trapani) resembles the size of cysts obtained for *A. salina* populations from Tarquinia and Sardinian salterns.

The multivariate procedure produces 12 discriminant functions for each analysis (males and females). In the case of the females, the first 11 functions significantly ($P \leq 0.01$) account for the increase of variance explained when they are included in the model. For males, the analysis needs only the first eight discriminant functions to completely separate the populations studied. These functions significantly ($P \leq 0.01$) account for the variance explained. However, in both cases, the four first discriminant functions account for the larger part of the variation (90.5% in the analysis pertaining to females and 93.2% in that for the males).

Figures 1 and 2 summarize the centroids (mean points for each population) for the first two discriminant functions obtained, for females and

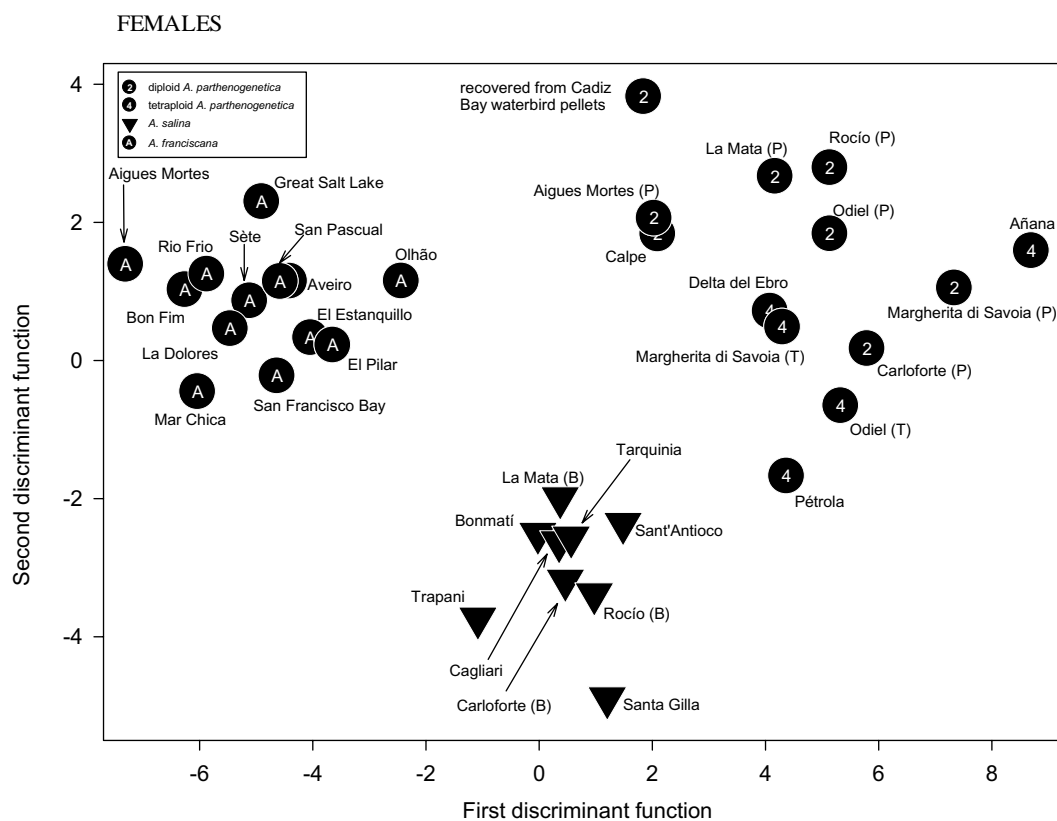


Figure 1. Group centroids of the populations studied for the first two discriminant functions resulting from the discriminant analysis on female morphometric variables.

Table 2. *Artemia* populations obtained in the laboratory from cyst samples collected in southwestern Spain, north of Morocco and southeastern France, and their relative specific composition where different species or strains appeared.

Locality	Sampling date	Specific composition
<i>Spain</i>		
Huelva Province		
Esteros Odiel saltern	06. 2002	<i>A. parthenog.</i> (d): 97% <i>A. parthenog.</i> (t): 3%
Cadiz Province		
N.S. del Rocio saltern	01. 2002	<i>A. parthenog.</i> (d): 98% <i>A. salina</i> (bisex.): 2%
El Estanquillo saltern	01. 2002	<i>A. franciscana</i>
El Pilar saltern	06. 2002	<i>A. franciscana</i>
San Pascual saltern	02. 2003	<i>A. franciscana</i>
La Dolores saltern	02. 2003	<i>A. franciscana</i>
<i>Morocco</i>		
Laguna Mar Chica saltern	06. 2000	<i>A. parthenog.</i> (d): 80% <i>A. franciscana</i> : 20%
<i>France</i>		
Sete-Listel saltern	05. 2002	<i>A. franciscana</i>
Aigues Mortes saltern	06. 2002	<i>A. parthenog.</i> (d): 2% <i>A. franciscana</i> : 98%
Fos saltern	05. 2002	<i>A. franciscana</i> (p. s.)
Pesquiers saltern	05. 2002	<i>A. franciscana</i> (p. s.)
Hyère saltern	05. 2002	<i>A. franciscana</i> (p. s.)
Thau Castelan saltern	05. 2002	<i>A. franciscana</i> (p. s.)

A. parthenog. (d): *Artemia parthenogenetica* (diploid); (t): tetraploid; (p. s.): alcohol preserved original specimens.

Table 3. *Artemia* cyst samples available from Italy.

Locality	Sampling date	Cyst diameter	Observations
<i>Veneto province</i>			
Comacchio salterns	1985	278	NH
<i>Apulia province</i>			
Margherita di Savoia salterns	1988	258	<i>A. parthenogenetica</i> (d): 67% <i>A. parthenogenetica</i> (t): 33%
Margherita di Savoia salterns	1988	267	<i>A. parthenogenetica</i> (d): 22% <i>A. parthenogenetica</i> (t): 78%
<i>Lazio province</i>			
Tarquini salterns	2002	243	<i>A. salina</i>
<i>Sicilia province</i>			
Isola Longa salterns	1985	245	NH
<i>Sardinia province</i>			
Cagliari salterns	1988	254	<i>A. salina</i>
Carloforte salterns	1987	251	NH
Carloforte salterns	1988	256	<i>A. salina</i>
San Antioco salterns	?	255	<i>A. parthenogenetica</i> (d) <1% <i>A. salina</i>
<i>ARC 579</i>			
Santa Gilla salterns	1994	253	<i>A. salina</i>

Mean diameter of cysts (μm) measured with a micrometer eyepiece. Relative specific composition where different species or strains appeared. (d): *Artemia parthenogenetica* diploid, (t): tetraploid.

NH: No hatching cysts. ARC 579: *Artemia* Reference Center cyst bank sample.

males, respectively. The populations analysed can be split into three different groups when females (Figure 1) are considered and two different groups for males. Two of the three groups obtained for females are shown to be quite homogeneous, and these include 13 *A. franciscana* and 9 *A. salina* populations. The third group, more complex and less homogeneous, includes diploid and tetraploid parthenogenetic populations.

When males are considered (Figure 2), only two groups are observed owing to the absence of males for parthenogenetic populations. The group concerning *A. salina* males looks more homogeneous, whereas the other group, dealing with *A. franciscana* males, shows a clear split between a group of males morphologically similar to those originally from Great Salt Lake (Utah, USA), whereas the others are similar to San Francisco Bay (California, USA) ones, suggesting the possibility that the different American brine shrimp populations introduced in the Western Mediterranean localities originate from cysts imported from both parts of the USA. These data also support the view of Pilla and Beardmore (1994) on the greater usefulness of male traits in this type of morphological analysis.

Discussion

The first recorded deliberate introductions of *Artemia franciscana* were those carried out on a Pacific Island and in Brazil in the 1970s (VanStappen 2002). According to our results obtained through the screening of old and updated brine shrimp cyst samples collected in salterns from various western Mediterranean countries, including the Atlantic shore salterns in Portugal and southwest Spain, the presence of *A. franciscana* as an exotic invasive species is confirmed in Portugal, Spain, France, as well as in the north of Morocco.

The presence of *A. franciscana* in the southwest of Portugal appears to date from the early 1980s as suggested by previous information (Hontoria et al. 1987). The American brine shrimp populations probably then spread (or was introduced) to the North, invading hypersaline environments in central Portugal, i.e., Sado and Tejo estuaries during the course of this decade, reaching the salterns in the Aveiro district at the end of 1980s or early 1990s and outcompeting autochthonous *Artemia* populations.

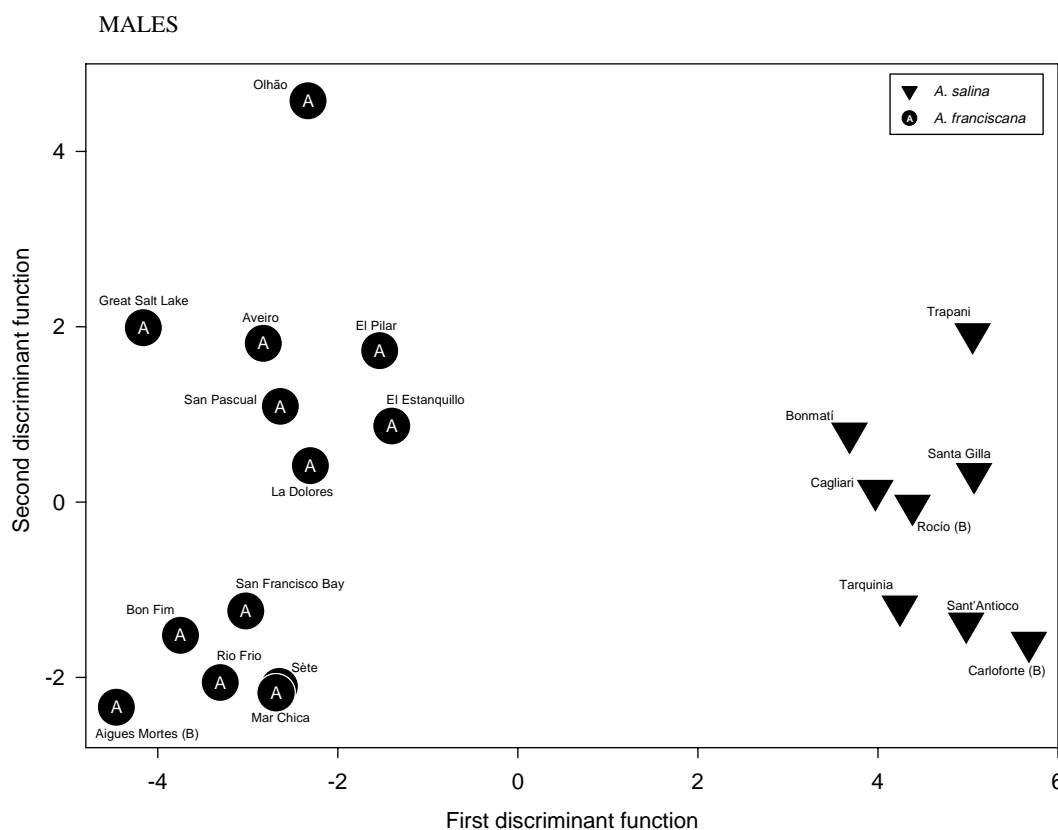


Figure 2. Group centroids of the populations studied for the first two discriminant functions resulting from the discriminant analysis on male morphometric variables.

The salterns in the southwest of Spain, especially in the provinces of Cadiz and Huelva, have been the subject of field studies over several decades. They provide a well-documented case of coexistence of parthenogenetic (diploid and tetraploid) strains and the amphygonic *A. salina* (Amat 1983a; Amat et al. 1995). However, in most of the 140 old salterns exploited around the Cadiz Bay (Puerto Real, San Fernando, Chiclana and Cadiz), salt extracting activity ceased at the beginning of the 1980s (Amat 1983b), their land use changing to aquacultural projects. This fact presumably provoked non-intentional inoculations of *A. franciscana* through hatchery effluents, while a few of them became the source of brine shrimp products for aquarists and pet markets after intentional inoculations (J.A. Calderon, pers. comm.).

The same aquacultural source for the introduction of American brine shrimp in the south of Spain and Portugal is presumably responsible for

its presence in the French Mediterranean (see below), where an unusual sexual population of *Artemia* was previously recorded by Thiery and Robert (1992). A close inspection of their paper shows that they had found an introduced population of *A. franciscana*, although they failed to name the species.

Italian samples studied from eight localities do not show the presence of *A. franciscana*. In these Italian salterns, only the autochthonous strains were found. This supports the results of Nascetti et al. (2003) in their study of the genetic structure of Italian *Artemia* from brackish and hypersaline waters.

According to Ehrlich (1984), Lodge (1993) and McMahon (2002), successful invasive species usually display different degrees of the following attributes: (1) abundance in their original range or large native range, (2) polyphagous or eurytrophic (i.e. wide feeding niche), (3) much genetic variability or phenotypic plasticity, (4) short

generation times, (5) fertilized females able to colonize alone (i.e. single parent reproduction), (6) vegetative reproduction, (7) larger than most related species, (8) high dispersal rate, (9) associated with human activities (human commensalism) and (10) able to function in a wide range of physical conditions.

In fact, the biological attributes of species are not the only reason of successful introductions. According to Williams (1996) and Blackburn and Duncan (2001), the match between the climatic or environmental conditions in a species natural range and the climate in the location of the introduction could also be an important factor.

There are many examples of invading aquatic species showing most of the cited traits, but not all these traits can play a definitive role in determining the success of invasions, the shifts in the structure of invaded communities over time, and the probability of extinction of autochthonous species. How well *Artemia* complies with most of these traits thought to characterize invasive species is addressed below.

It has been argued that there is little evidence suggesting that physiological capacity to tolerate and function in a wide range of physical conditions is a prerequisite to successful invasions of aquatic habitats (McMahon 2002). In the genus *Artemia*, although the information on the tolerance of its different species to a wide range of physical conditions is not complete, it is commonly accepted that its adaptation to the severe habitats of hypersaline ecosystems, and its wide distribution in all continents except Antarctica, mean that the species and populations can withstand the widest salinity and temperature ranges among aquatic organisms, living in salinities at or below seawater concentration (35 g l^{-1}), up to saturation level (300 g l^{-1}).

It is possible to assimilate the filter feeding mechanism of *Artemia* to a polyphagous regime equivalent to eurytropy, provided that the filtered particles do not exceed a range of critical sizes, i.e., between 6.8 and $27.5 \mu\text{m}$ (Gelabert 2001).

The sympatry of sexual and asexual autochthonous *Artemia* species recorded in several sites, e.g. Spanish Mediterranean coastal salterns (Browne and McDonald 1982; Amat 1983a; Browne et al. 1988), or lake Urmia (Iran) and peripheral hypersaline lagoons (N. Agh, pers.

comm.) has previously motivated laboratory studies of the interaction between sexual and asexual populations. These competition studies can shed light on life history traits that may explain the success of *A. franciscana* as an invader.

Competition experiments between bisexual and asexual *Artemia* populations from several localities of the Old World have been carried out in the laboratory (Browne 1980; Browne and Halanych 1989), with *A. franciscana* usually incorporated as a model species since it is the best known and studied taxon (Lenz and Browne 1991). In these experiments, *A. franciscana* populations outcompeted parthenogenetic (diploid) populations in 91% of the scorable trials. However, when these *A. parthenogenetica* populations competed against the co-occurring Old World and Mediterranean sexual populations *A. salina*, bisexuals were eliminated in 98% of the trials. Thus, the competitive abilities of the *Artemia* populations under the experimental conditions tested are *A. franciscana* > *A. parthenogenetica* > *A. salina*. However, salinity, temperature and other environmental gradients are likely to influence the relative performance of each species.

Many invasive aquatic species with significant ecological impacts are characterized by adaptations supporting rapid population growth, including rapid individual growth, early maturity, short generation times, high fecundities and small egg-offspring size. These are traits characteristic of species adapted to unstable habitats, with frequent population density reductions or disappearance associated with unpredictable natural environmental events (Browne and Wanigasekera 2001). *Artemia* and especially *A. franciscana* can be considered to exhibit these traits, *A. franciscana* being a more extreme *r*-strategist (Lodge 1993; Williamson 1996) compared to the species it outcompetes.

A wide variety of factors such as environmental cues, life-history traits, heterozygosity levels and genetic variability may contribute in determining the competitive abilities of the *Artemia* populations. These variables cannot be pooled together in experimental designs, but the partial information available from studies of each factor and the evidence from studies in the field where competition occurs suggest the possibility of competitive exclusion of native *Artemia* by

A. franciscana in 10–100 generations (Miller 1967), although according to Lenz and Browne (1991) it may be attainable in two or three generations.

Most *Artemia* species reproduce primarily by ovoviviparity under favourable environmental factors, especially when there is no food limitation, but they switch to oviparity (producing cysts that can undergo prolonged diapause and cryptobiotic periods) when unfavourable environmental factors threaten the ovoviviparous reproduction and the population persistence. These cysts are the best way to ensure the appearance of a new population under renewed favourable conditions promoting cyst hydration and hatching, i.e. the renewal of the population in the following season. These cysts are also the best way to ensure a successful dispersion of *Artemia* populations. These cysts are thought to be broadly dispersed by wind transportation among short distances, or by birds for longer distances (Figuerola and Green 2002; Green et al. 2002).

Last but not the least, the presence of *A. franciscana* from the New World in the Western Europe and Mediterranean shores is unquestionably associated with a human activity, with the aquaculture of marine species of commercial interest. With the unavoidable development of hatcheries to obtain postlarvae and fingerlings for aquaculture, the use of *Artemia* nauplii as a diet for larval culture became widespread. Dormant cysts of *Artemia* can be stored for long periods in cans and then used as an off-the-shelf food requiring only 24 h of incubation to obtain live nauplii (Lavens and Sorgeloos 2000). From the early 1950s, commercial sources of cysts initially originated from the coastal salterns in the San Francisco Bay, California, USA, and the inland Great Salt Lake, Utah, USA. These cysts were primarily marketed for the aquarium pet trade, and in the mid-1970s, the demand increased from emerging aquaculture operations, currently attaining requirements of about 2000 metric tons of cysts annually.

Shrimp culture, based on Penaeids, started to develop rapidly in the Mediterranean area (mainly in Italy and France) in the latter years of the 1970 decade (Lumare 1990). Experimental productions of *Penaeus japonicus* in the southwest of Spain (Cadiz) started in 1982, and in 1986, about 20 million postlarvae were reared

(Rodriguez 1986). Shrimp culture was largely replaced by marine finfish (sea bass, sea bream) culture in the early 1980s, when many old seaside salterns that had been traditionally exploited in the area of Cadiz bay switched their activity to intensive fish culture. By 1998, 900 ha of old saltern ponds had been converted to intensive fish culture (Espinosa et al. 1999).

In southwestern Portugal (Algarve province), old salterns were converted to prawn and fish culture in 1985–1986 (Gouveia 1994), but American brine shrimp may have been introduced earlier for the aquarium pet trade (M.N. Vieira, pers. comm.).

In the early 1970s, there were important advances in sea bass intensive cultures in pilot plants settled in the area of Sete (Languedoc, France) near coastal lagoons and marine salt exploitations (Barnabe 1974a, b). During this decade, several fish farms developed in the lagoons and brackish environments along the Languedoc shore: Salses-Leucate, Thau., etc.

These aquaculture developments have been closely linked to the success in shrimp and fish hatchery productions, where larviculture relied on the supply of live food organisms in sufficient quantity. To date, these living preys are rotifers (*Brachionus plicatilis*) obtained through season-round massive culture, and *Artemia* nauplii from the massive hatching of cysts purchased in the international market from sources in America, especially from Great Salt Lake, Utah.

Our results suggest that the native populations of *Artemia* in the Mediterranean region are under severe threat from competition with the expanding *A. franciscana*. In an attempt to prevent or slow down further spread of this exotic species, we suggest that aquaculture activities should be subject to tighter regulation. Where possible, the use of cysts from native species should be encouraged.

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