

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

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16 Abstract

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

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36 **Keywords:** *Artemia*, invasion, toxicity, chlorpyrifos, life tables, AChE

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 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 areas of scientific knowledge, 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 conditions (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 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

1 populations used in the bioassays. Work performed by Browne (1980), Sarabia et al. (2002) or Varó et al.
2 (1998) showed consistent distinct patterns of response to heavy metals or pesticides, according to
3 different species or strains for testing purposes.

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

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

26 27 **2. Materials and Methods**

28 29 2.1. Experimental organisms and conditions

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

37 Original brine shrimp cysts collected in 1988 for La Mata PD and in 2008 for "La Trinitat" AF,
38 and kept at the "Instituto de Acuicultura Torre de la Sal" (IATS) cyst bank, were used. These cysts were
39 hatched in seawater (35 g/l) at 28 °C under 24h 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 sufficient 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, 12h:12h 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 48-72h actual concentrations of chlorpyrifos in water were less than 10% from nominal values (Varó et al. 2000; 2002b) 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 developing this study, brine and living *Artemia* biomass samples, collected seasonally over one year (2007) in “La Trinitat” saltworks, were analysed 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. The sublethal chlorpyrifos concentrations used were based on previous *Artemia* studies carried out in our laboratory (Varó et al. 1998; 2000).

2.3. Life table experiments

To study the life-history responses of AF and PD to chlorpyrifos a total of 20 females showing signs of vitellogenesis were isolated individually, together with a male in the case of the bisexual AF, in 50 ml polypropylene corning tubes filled with each of the selected chlorpyrifos concentrations, plus the controls. Animals were kept under the same conditions of temperature, salinity and photoperiod, and fed

1 as indicated above. Their survival and reproduction were examined every 2-3 days. Afterwards, the
2 culture medium was renewed with the appropriate nominal concentration of the pesticide and microalgae.
3 Dead specimens were removed and replaced during the first 3 days only. Afterwards, stock cultures were
4 kept under the same experimental culture conditions. The experiment end point was established after
5 obtaining ten broods per female or after the death of all individuals (male or female).

6 To assess the effect of sublethal concentrations of chlorpyrifos on the biological fitness of the
7 AF and the PD *Artemia* species, life table experiments were carried out and the following life table traits
8 were examined: (1) temporal parameters: pre-reproductive period (PRP), defined as the time taken to
9 obtain the first brood; period between broods (P/B), defined as time period (in days) elapsed between
10 successive broods. (2) Quantitative parameters (fecundity): total number of broods per female (B), total
11 offspring per female (OFF), total offspring per brood (OFF/B). (3) Type of reproduction: ovoviviparous
12 (nauplii) (OVO) versus encysted (cysts) (OVIP). The quality of reproduction was assessed in the case of
13 the ovoviviparous offspring, through the percentage of viable nauplii (VN) and percentage of dead nauplii
14 plus abortive embryos (NVN). For encysted offspring, the percentage of viable and non-viable cysts (VC
15 and NVC, respectively) was also calculated.

16 17 2.4. ChE activity

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19 The effect of chlorpyrifos on ChE activity was evaluated in metanauplii (AF: 6 days, PD: 5
20 days), juveniles (AF: 9 days, PD: 14 days) and adults (AF: between 16 and 21 days, PD: between 21 and
21 28 days) exposed to the selected pesticide concentrations in the stock cultures and kept in the conditions
22 described above. Once the animals reached the developmental stage desired, 10 individuals were sampled
23 and stored individually at -80°C until enzymatic analyses were performed.

24 For enzymatic analyses one metanauplii, juvenile or adult was manually homogenised in 350-
25 500 µl of ice-cold phosphate buffer (100mM), using a microcentrifuge tube pestle. The homogenates
26 obtained from metanauplii and juveniles were used directly for enzymatic determinations, whereas those
27 from adults were first centrifuged at 10.000g for 30 sec, to remove the biggest remains of tissue, and the
28 resulting supernatant was used for enzyme activity. ChE was assayed by the Ellman method (Ellman et al.
29 1961) following the procedure described in Varó et al. (2002a) using ATC as substrate, with some
30 modifications. Briefly, 0.1 ml of homogenate was mixed with 0.2 ml of the reaction solution (30 ml of
31 phosphate buffer 100 mM, pH= 7.2, 1mL DTNB 10 mM plus 0.2 ml ATC 75 mM) and absorbance was
32 read at 415 nm. The activity of ChE was measured in kinetic mode using a TECAN Ultra Evolution
33 microplate reader at 25 °C for 15 min, after an incubation period of 15 min. Activity was expressed as
34 units (U = nmol of substrate hydrolysed per minute) per mg of protein. Total protein content of the
35 samples was determined by the Bradford method (Bradford 1976) adapted to microplate, using the
36 Bradford Bio-Rad Protein Assay and BSA as standard. The absorbance was read at 595 nm. All enzyme
37 analyses and protein determinations were carried out in triplicate per sample.

38 39 2.5. Data analyses

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1 The effects of chlorpyrifos on survival during the life table experiments were analysed using the
2 logrank (Mantel-Cox) test for multiple comparison of survival curves (Bland and Altman 2004). P values
3 were corrected with the Bonferroni method by simultaneously comparing all pairs of curves. Data were
4 tested for normality and homogeneity of variance with Kolmogorov–Smirnov and Levene tests,
5 respectively. The reproductive parameters showing homogeneous variance were compared by one-way
6 ANOVA, followed by *post hoc* Tukey multiple comparison test. When heterocedasticity was found,
7 differences were checked by the Brown-Forsythe test followed by the Games-Howell test for multiple
8 mean comparisons (Amat et al. 2007). To further process in an integrative manner the results of the life
9 table experiments and explore the relationship within the recorded set of life table traits, each variable
10 from the life table was log-transformed and introduced in a multivariate principal component analysis
11 (PCA) using VARIMAX rotation. Score plots were further generated as a potential pattern recognition
12 tool using the species and the toxic concentrations as labels. Prior to performing PCA, the suitability of
13 data was assessed by the Kaiser-Meyer-Olkin method and Bartlett test of sphericity (Ruiz et al. 2000).

14 An age-structured matrix model was used to calculate the finite population growth rate (λ) for
15 each species and treatment. Sukumaran and Grant (2013) have successfully used this kind of analysis to
16 explore the relative sensitivities of these two species to genotoxins. Therefore, age-specific fertilities (F_i)
17 and survival probabilities (P_i) were calculated from life table data (see section 2.3) on 5-day intervals and
18 a Leslie matrix was built up for each species and treatment. There were 12 age classes for AF (up to a
19 maximum lifespan of 55-60 days) and 15 age classes for PD (up to a maximum lifespan of 70-75 days).
20 Because cysts do not contribute immediately to population growth rate, F_i values were computed on the
21 numbers of viable nauplii observed only. All calculations were based on the birth-flow equations from
22 Caswell (2001).

23 The finite population growth rate (λ) was calculated as the dominant eigenvalue of each Leslie
24 matrix. Then, we conducted a decomposition analysis (De Kroon et al. 1986; Caswell 2001) to determine
25 the differences and contributions of age-specific P_i and F_i on λ between treatments and controls,
26 separately for each species. Accordingly, we used the control (C) matrices of each species as a baseline
27 against which to measure the effects of increasing levels of chlorpyrifos and the effect of the solvent
28 (CA). Matrix population models were run and analyzed using the popbio package (Stubben and Milligan
29 2007) in R version 3.0.1 (R Development Core Team 2013).

30 ChE data were also tested for normality and homogeneity of variance with Kolmogorov–
31 Smirnov and Bartlett tests, respectively. Deviations from normality were corrected with log ($x+1$)
32 transformation. The effect of chlorpyrifos concentrations on ChE activity for each development stage was
33 assessed using one-way ANOVA, followed by *post hoc* Dunnett comparison test to determine significant
34 differences among the concentrations of pesticide assayed relative to the control group. One-way
35 ANOVA and *post hoc* Tukey multiple comparison tests were also used to determine differences in normal
36 ChE activity of non-exposed animals. A t-test was used to compare the normal range of activity in non-
37 exposed individuals for each developmental stage of the two species. The ChE activity data were further
38 analysed multifactorially by General Linear Model to assess the effect due to toxicant concentration,
39 developmental stage and sex (for the bisexual AF strain only). The partial eta-squared statistics describing
40 the proportion of total variability attributable to each factor was computed. Significant differences were

1 established at $P < 0.05$. Results are presented as means \pm SD (standard deviation). Statistical analyses
2 were carried out using the SPSS 20 software package and the Prism software for MacOS X.

3 4 **3. Results**

5 6 3.1. Effect of chlorpyrifos on life table parameters

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8 The effects of chlorpyrifos on survival during life table experiments are shown in Fig. 1. The
9 results of the logrank test for multiple comparisons of survival curves reveal a significant effect of the
10 pesticide concentration on the survival of both *Artemia* species. The highest concentration of toxicant
11 tested (5 $\mu\text{g/l}$) produced significantly higher mortality in adults of AF, as compared to the rest of the
12 treatments with exception of the solvent control group (CA). In these experimental groups, 50% and 60%
13 of the individuals survived respectively to the 24th day, while approximately 60% of the individuals
14 survived to the 30th day in the rest of the experimental groups, including the control.

15 By contrast, PD *metanauplii* exposed to 5 $\mu\text{g/l}$ of chlorpyrifos reached 100% mortality after 7
16 days. The survival of the individuals exposed to 1 $\mu\text{g/l}$ showed significant differences with respect to the
17 rest of the groups. Fifty per cent of the individuals survived to the 8th day, whereas 100% mortality was
18 reached after 24 days (see Fig. 1).

19 There was an effect of the acetone treatment on the survival of the two species. Although the
20 survival obtained for this treatment followed a similar trend for both AF and PD, the differences respect
21 to the control (C) were more apparent in AF due to the higher survival of the latter (Fig. 1).

22 In general, most of the reproductive parameters measured in both *Artemia* species (AF and PD)
23 were not affected by toxicant exposure (Tables 1 and 2). In AF, chlorpyrifos concentration significantly
24 affected the fecundity parameters (B, OFF) and the amount of both encysted offspring (VC, NVC). In PD,
25 significant effects were found for temporal parameters (P/B), as well as for fecundity (B, OFF and
26 OFF/B). AF females presented shorter pre-reproductive periods (PPR) and similar periods between
27 broods (P/B) when compared to PD females. On the contrary, the fecundity of AF females, in terms of
28 total offspring per female (OFF) and total offspring per brood (OFF/B), was higher. Regarding the type of
29 reproduction, both *Artemia* species displayed a higher percentage of encysted offspring (OVIP), except at
30 the highest chlorpyrifos concentration tested (1 and 5 μl for PD and AF, respectively), where equilibrium
31 between oviparism (OVIP) and ovoviviparism (OVO) was found. In AF females, exposure to increasing
32 concentrations of chlorpyrifos caused higher percentages of non-viable offspring (NVN and NVC), and
33 exposure to 1 $\mu\text{g/l}$ chlorpyrifos produced significantly lower percentage of encysted offspring (VC and
34 NVC). On the contrary, exposure to chlorpyrifos did not significantly affect either the type or the quality
35 of reproduction of PD females.

36 PCA revealed three components with eigenvalues exceeding 1, which explained 81% of total
37 variance, with the first two components accounting for 61% of total variance (34% first, 27% second).
38 These first two components were used to ascertain relationships among variables. The component plot
39 (Fig. 2A) shows a clear association of reproductive parameters (OFF/B, OFF and B) on the positive side
40 of the first component, clearly opposed to temporal parameters (PRP and P/B) on the negative side. Thus

1 the first component could be associated with reproductive success or fertility. Variables OVIP and VC
2 were associated to the negative side of second component. They opposed NVN, OVO and VN, although
3 these variables were less associated with the positive side. NVC was loosely associated to both
4 components. Thus, the second component could be generally identified with the type of reproduction.

5 The score plots revealed a clear separation of the cases corresponding to the two *Artemia* species
6 on the first component (Fig. 2B), whereas no clear separation was found among the cases according to the
7 effects of the different pesticide concentrations (Fig. 2C). AF was clearly associated to the fecundity
8 variables.

10 3.2. Population level effects of chlorpyrifos and decomposition analysis

12 Population growth rate (λ) was substantially reduced from the control in the rest of treatments
13 (CA, 0.1, 1 and 5 $\mu\text{g/l}$ chlorpyrifos) in both species (Table 3). PD had a lower finite growth rate in all
14 cases, and suffered a higher reduction at increasing levels of chlorpyrifos than AF.

15 The effects of chlorpyrifos on fertility (F_i), and the contributions of these effects for both species
16 are shown in Fig. 3A and 4A, respectively. Fertility declined with increasing chlorpyrifos concentration
17 in both species, as reflected by the negative values of differences observed. Positive values observed at
18 advanced age classes in AF (from CA to 5 $\mu\text{g/L}$ chlorpyrifos) and in PD (only in CA) may represent a
19 delay in reproduction due to the toxicant effect. In AF, such a positive difference was greater at higher
20 chlorpyrifos concentration. Fertility differences over age 25-30 in AF, and later over age 35-40 in PD,
21 had no impact on λ as evidenced by contributions. Most of the impact of chlorpyrifos on fertility occurred
22 in the range 5-25 days of life in AF, while the range moved upwards to 25-40 days in PD.

23 The effects of chlorpyrifos on survival probabilities (P_i), and the contributions of these effects
24 for both species are shown in Fig. 3B and 4B, respectively. Survival and fertility are measured on
25 different scales, but their contributions to effects on λ are all expressed on the same scale, and thus are
26 directly comparable. Chlorpyrifos had almost no effect in survival in AF at concentrations up to 1 $\mu\text{g/l}$. At
27 5 $\mu\text{g/l}$, it reduced survival probability, especially at later ages. Notwithstanding, the contributions of these
28 effects were negligible. Hence, the finite growth rate in AF was more sensitive to changes in fertility than
29 in survival. On the other hand, chlorpyrifos affected negatively survival probability in PD at all
30 concentrations, especially at older ages. Nonetheless, only the effects during the first 25 days of life make
31 any contribution to the observed reduction in λ when PD was exposed to 1 $\mu\text{g/l}$ chlorpyrifos.

33 3.3. ChE activity

35 The effect of increasing concentrations of chlorpyrifos on ChE activity of metanauplii, juveniles
36 and adults of AF and PD are presented in Fig. 5 and 6, respectively. For AF, adult females exposed to
37 chlorpyrifos showed a significant reduction of ChE activity at 1 and 5 $\mu\text{g/l}$, whereas in males, the ChE
38 activity of both juveniles and adults significantly decreased in all concentrations tested.

39 Exposure to 1 and 5 $\mu\text{g/l}$ of chlorpyrifos resulted in significant decreases in ChE activity in PD
40 metanauplii. Since metanauplii died after 7 days of exposure at the highest pesticide concentration (5

1 µg/l) no samples of juveniles and adults were available for ChE activity determinations in this species.
2 However, significant decreases of the ChE activity were found in juveniles and adults exposed at 1 µg/l.
3 No significant effect of the solvent (acetone) on ChE activity was observed (see Fig. 6).

4 The range of normal ChE activity on non-exposed AF and PD individuals is shown in Fig. 7.
5 The mean ChE activity values found for AF were 16.31 U/mg protein for metanauplii, and 11.99 and 8.62
6 U/mg protein for juvenile males and females respectively. Corresponding values for adults were 6.22 and
7 5.33 U/mg for males and females respectively. For PD, normal ChE activity was 12.56 U/mg protein for
8 metanauplii, 7.99 U/mg protein for juveniles, and 4.25 U/mg protein for adults. Normal ChE activity
9 significantly decreased from metanauplii to adults in both *Artemia* species with the exception of the
10 juvenile AF males. Moreover juvenile AF females, and adult AF females and males, did not display
11 differences in normal ChE activity, whereas for juvenile and adult stages of PD, significant differences
12 were found.

13 The multifactorial analysis of the ChE activity of AF showed significant effects for sex, toxicant
14 concentration and developmental stage, but the interactions were not significant. Partial eta-squared
15 analysis revealed that variability was mainly explained by the toxicant concentration (eta squared =
16 0.972), followed by sex (eta squared = 0.900) and development stage (eta squared = 0.873). For PD, the
17 multifactorial analysis of the ChE activity (including metanauplii) revealed a significant effect of the
18 factors' toxicant concentration and developmental stage, as well as the interaction. The variability was
19 explained similarly by both factors (eta squared = 0.778 and 0.710 for development stage and toxicant,
20 respectively), followed by the interaction whose contribution was clearly inferior (eta squared = 0.356).

21 22 **4. Discussion**

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24 Life history traits that make species more invasive have been of continuing interest because of
25 their potential predictive power. Studies on NIS have focused on fitness traits that may predispose species
26 to rapid population expansion (Sakai et al. 2001), i.e. traits related mainly to r-selected life histories, like
27 pioneer use of habitat, short generation time, high fecundity and high growth rates (see Kolar and Lodge
28 2001). Some authors (O'Connor 1986; Newsome and Noble 1986) assign additional abilities to NIS, such
29 as increased dispersal mechanisms, ability to compete with native species for resources, repeated
30 introductions, tolerance to a broad range of environmental conditions, or ability of females to colonize
31 alone and in association with humans (Ehrlich 1989).

32 Focusing on Mediterranean aquatic hypersaline environments and their conspicuous native brine
33 shrimp species and strains, it would be logical to assign some of the fitness abilities exposed above to the
34 native diploid *A. parthenogenetica*. Under temperate conditions, usual in the Mediterranean basin,
35 parthenogenetic diploid brine shrimp strains show physiological responses and demographic traits
36 theoretically superior when facing competition with Mediterranean and Old World bisexual species
37 (Amat 1983; Browne and Halanych 1989; Browne 1992; Barata et al. 1996; Browne and Wanigasekera
38 2000). But whilst in general, parthenogenetic reproduction associates to very effective and rapid dispersal
39 and colonization mechanisms, the real picture shows the American bisexual *A. franciscana* going beyond
40 its original range and reaching the Old World, spreading from western (Amat et al. 2005) to eastern (Van

1 Stappen et al. 2007) geographical extremes. Under the same climatic conditions prevailing in the
2 Mediterranean area, this allochthonous species has been able to develop improved physiological
3 responses and demographic traits comparatively to bisexual and parthenogenetic native species and
4 strains, as several authors have experimentally demonstrated (Varó et al. 2000; Sarabia et al. 2002;
5 Browne and Wanigasekera 2000; Browne et al. 2002; Amat et al. 2007), ultimately becoming an invasive
6 NIS.

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7 The research reported here is based on the utilization of the American brine shrimp species
8 introduced and established in “*La Trinitat*” saltworks. To date, this seems to be the only hypersaline
9 ecosystem invaded with this NIS in the Spanish western Mediterranean. Prawn culture was developed in
10 these saltworks during the 1980s of the last century (Amat et al. 2007), with the cysts from this species,
11 usually from the original population at Great Salt Lake (Utah, USA), being the natural resource “*brine*
12 *shrimp cysts*” most widely available in the global market trade for marine aquaculture (Lavens and
13 Sorgeloos 2000).

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14 *Artemia* populations inhabiting saltworks in the western Mediterranean area are exposed to a
15 variety of contaminants, which may produce an appreciable toxic stress affecting their survival and
16 reproduction. These compounds seem to be ubiquitous in the biotic compartment of aquatic environments
17 and have been detected in *Artemia* specimens in previous works (Wang and Simpson 1998). The
18 contamination pattern observed in marine saltworks along the Mediterranean coast shows a great variety
19 of organic compounds, with around a dozen of them (including chlorpyrifos) repeatedly present (Serrano
20 et al. 2011; 2012). Sources of these contaminants are certainly run offs from industries, farms and
21 urbanized areas. According to Serrano et al. (2011; 2012 and bibliography therein) a substantial presence
22 of pollutants in hypersaline environments is expected. They can be concentrated during the formation of
23 brines, and then persist throughout the crystallization process. Among these pollutants the
24 organophosphorous pesticide chlorpyrifos is the only contaminant detected in both water (seawater,
25 brines) and in *Artemia* biomass in “*La Trinitat*” saltworks (Serrano et al. 2012). Non-polar
26 organochlorine contaminants, pp’DDE and PCBs, were also detected in *Artemia* specimens in these
27 saltworks, but not in brines due to their lipophilicity. All these pollutants can be recognized as potential
28 threats to vulnerable populations inhabiting the waters of saline ecosystems.

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29 Varó et al. (1998; 2000; 2002b) reported that different *Artemia* strains show different degrees of
30 sensitivity to toxicants, including organophosphorus and organochlorine compounds, with *A. franciscana*
31 being one of the most resistant forms. Can this exposure to toxicants act as one of the forces driving the
32 success of the NIS *A. franciscana* over the native *Artemia* species, particularly over the diploid
33 parthenogenetic strain?

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34 The results of the life table experiments for survival and reproduction reported here for both
35 *Artemia* species are in line with those reported in the literature, in that they indicate differences in the
36 sensitivity of the nauplii to acute exposure to organophosphate pesticides, both among species and within
37 strains of the same species (Varó et al. 1998; 2002a). The same is true for metals such as Cd (Sarabia et
38 al. 2002). A decrease in survival after acute and chronic exposure to chlorpyrifos has also been found in
39 different species of *Daphnia* (Palma et al. 2009; Zalizniak and Nugedoda 2006). Our results also show
40 that survival was more sensitive than reproduction to chlorpyrifos exposure, as observed for these same

1 two species when exposed to other toxicants (e.g. EMS; Sukumaran and Grant 2013). However, the
2 response of both species in terms of sensitivity to chlorpyrifos was different, since mortality was higher in
3 PD in all concentrations tested. This was reflected in higher reduction percentages on population growth
4 rate at all concentrations tested in PD with respect to AF. It is worthy of note that at 5 µg/l of
5 chlorpyrifos, all animals died before the juvenile stage was reached. Moreover, survival in PD strain
6 exposed to 1 µg/l of chlorpyrifos gradually decreased with time until total mortality was reached at 24
7 days. At this time, the survival of AF exposed to the same concentration was 80 %.

8 The unavoidable effect of carrier solvent in aquatic toxicology studies has been well
9 acknowledged (Rufli et al. 1998, Hallare et al. 2006, Marquis et al. 2006, Hutchinson et al. 2006). It is
10 worth to note that even though in our study this solvent effect is also present, and quite apparent in CA
11 treatment, the highest acetone concentration used (5 µg/l) was well under the maximum OECD
12 recommendations (79 mg/l). In both species, the addition of the pesticide at the highest concentration,
13 increased the mortality respect to the CA group, especially in PD where total mortality was reached thus
14 highlighting the effect of the pesticide over the effect of the carrier.

15 The chronic effects of chlorpyrifos on the reproduction process affected the fecundity of both
16 *Artemia* species. Also, in PD a reduction in the number of offspring (OFF) was observed at exposure
17 concentrations of 1µg/l. Moreover, changes in the quality of reproduction were found in AF exposed to 1
18 and 5 µg/l of chlorpyrifos, causing a tendency to produce more non-viable offspring, while in PD the
19 period between broods (P/B) was affected by exposure to 1 µg/l of chlorpyrifos. These results are in line
20 with those described previously for freshwater invertebrates, mainly daphnids, in which a decrease in the
21 offspring production has been reported at concentrations of chlorpyrifos ranging from 0.005 to 0.5 µg/l
22 (Naddy et al. 2000; Zalizniak and Nugegoda 2006; Palma et al. 2009). On the contrary, Printes et al.
23 (2008) did not find a direct effect on life history traits in *D. magna* exposed to the organophosphate
24 insecticide acephate. All other reproductive parameters studied were not affected by chlorpyrifos
25 exposure in both *Artemia* species, at least in the sense that no clear concentration-response relationships
26 were observed.

27 The results also indicated notable differences in tolerance to chronic exposure to chlorpyrifos
28 between both *Artemia* species. Although, both showed an elevated tolerance to the high range of pesticide
29 tested, AF survived better and its fecundity was less affected by the exposure to the pesticide than PD. In
30 fact, the lowest concentration tested (0.1 µg/l) did not produce changes in survival and reproduction
31 compared with the control. To put these facts into context, it should be borne in mind that 0.1 µg/l is the
32 maximum allowed concentration (MAC) in surface water proposed for chlorpyrifos by the European
33 Community (EEC 2007) in environmental quality standards (EQS). This concentration should provide
34 protection of the aquatic ecosystem against short-term exposure. The annual average quality standard
35 concentration (AA) is the limit established for protection against chronic exposure in EQS. For
36 chlorpyrifos, the AA has been established at 0.04 µg/l, which is 25 fold lower than the concentration
37 producing negative effect in survival and reproduction in PD (1 µg/l), and 125 fold lower than the
38 concentration that affects AF in this study (5 µg/l). In general, the life history traits in AF were
39 characterized by shorter pre-reproductive periods and higher reproductive outputs, in terms of fecundity
40 and offspring quality, than those showed by PD, confirming the finding of Amat et al. (2007) for different

1 populations and species of *Artemia*. Consistently, PCA analysis of the reproductive parameters revealed a
2 clear separation by *Artemia* species, whereas exposure to toxicant had not a clear effect in this separation.

3 The results of our decomposition analysis allow the examination of how much of the change
4 caused by chlorpyrifos on the finite growth rate can be attributed to survival and how much to
5 reproduction. From our results, it follows that all demographically important effects of chlorpyrifos
6 occurred at early ages in both species, but in a different manner for each. Chlorpyrifos had sizable effects
7 on survival and fertility at advanced ages in all concentrations tested for both AF and PD, however these
8 effects had negligible impact on their respective population growth rates. Chlorpyrifos reduced
9 population growth rate in AF by reducing fertility during the first 35 days of life, as evidenced by the
10 differences plots (Fig. 3A, left panels). The contributions of age-specific fertilities to population growth
11 rate (Fig. 3A, right panels) were higher than age-specific survival probabilities, which were not affected
12 by chlorpyrifos in this species (Fig. 3B, right panels). On the other hand, the population growth rate in PD
13 was more affected by the effects of chlorpyrifos on age-specific survival probabilities than on fertilities,
14 the effects being noticeable at 1 µg/l of chlorpyrifos, but fatal at the highest concentration tested.

15 It is worthy to remind here that we did not consider the production of dormant cysts for the
16 computation of age-specific fertilities, as these propagules do not necessarily contribute to current
17 population growth rates. Sukumuran and Grant (2013) pointed out that considering cysts for the
18 computation of fertilities causes inflated values of population growth rates, although these authors also
19 stressed the role of cysts in long-term persistence of these populations in ephemeral waterbodies.
20 Furthermore, Varó et al. (2006) have shown that the chorion of *Artemia* cysts acts as a barrier slowing
21 down the entry of chlorpyrifos into the embryo, and that chlorpyrifos has little effect on hatching. Here,
22 we opted for being conservative because direct hatching rates of AF and PD cysts exposed to the
23 chlorpyrifos concentrations assayed in this study were not available for each age class, so we could not
24 reliably estimate the numbers of nauplii hatched from cysts contributing to age-specific fertilities.

25 The ChE activity was affected after chlorpyrifos exposure in both *Artemia* species, which
26 confirmed the relevance of ChE as biomarker of exposure in invertebrates, as shown in previous studies
27 on *Artemia* and *Daphnia* (Varó et al. 2002a; Barata et al. 2004). Differences in ChE sensitivity to
28 chlorpyrifos were found between developmental stages. The ChE activity of juveniles and adults of both
29 species was lower than in metanauplii, with the exception of the AF juvenile females that did not show
30 significant inhibition of the enzyme activity in any of the concentrations tested (see Fig 5). Our results
31 show that exposure to the highest chlorpyrifos concentrations (1 and 5 µg/l) led to a reduction of up to 80
32 and 85 % of the ChE activity for adults of AF and PD, respectively, before death occurs. It is interesting
33 to stress that, although in more reduced numbers as compared to that of the control, both species are able
34 to produce offspring (Table 1 and 2). In the results of a previous study (Varó et al. 2002a), similar levels
35 of ChE inhibition were obtained for nauplii of the same *A. parthenogenetica* strain and for the bisexual *A.*
36 *salina*, after 24 h of exposure to their median lethal concentration (LC50) values of chlorpyrifos without
37 lethal effects. No differences in ChE activity in non-exposed individuals for the same stage of
38 development were obtained between both *Artemia* species. An inverse relationship between ChE activity
39 and developmental stage has been found, coinciding with the results of previous studies carried out with
40 marine crustaceans and fish by Solé et al. (2006). These authors found a negative correlation between

1 AChE activity and size in Hake (*Merluccius merluccius*), and in Norway lobster (*Nephrops norvegicus*).
2 A similar inverse relationship between body size and acetylcholinesterase (AChE) has been described in
3 *D. magna* (Printes et al. 2003).

4 Most probably the invasibility triggered by the American brine shrimp is fairly supported by its
5 association to human activities, especially marine aquaculture and pet trade. These anthropic
6 interventions tend to strengthen the role of propagule pressure, provoking and supporting the invasion.
7 They are related to the number of releases and/or the number of individuals released in a fit environment.
8 If these increase, propagule pressure also increases, determining the successful establishment of a NIS
9 (Lockwood et al. 2005). Besides, the successful invasion by the sexual species may be favored by a
10 combination of high genetic diversity, high fitness, persistence in the cyst bank, and putative presence of
11 negative factors affecting the parthenogenetic native populations (i.e. presence of toxicants). Although
12 our results indicate a high tolerance to chronic exposure by chlorpyrifos of both *Artemia* species, the
13 features achieved by bisexual reproduction may allow advantages to persist that, in harsh environments
14 such as hypersaline biotopes polluted with xenobiotics, change into specific adaptations leading to
15 maximizing individual survival, for example through the r-strategy. As a consequence of this selective
16 pressure, sexual organisms reproduce quickly and in great numbers, developing shorter maturation times,
17 even with lower nutrient requirements, as opposed to their asexual counterparts (Lin and Lin 2010). Such
18 a combination of rapid population growth with the maintenance of genetic diversity, typically eroded in
19 parthenogenetic species (Butlin 2002, Mergeay et al. 2006), would explain invasion success in bisexual *A.*
20 *franciscana*. Interestingly, a similar mechanism has been proposed to explain the resistance to invasibility
21 in a population of sexual *Daphnia pulex* against asexual clones of the same species (Innes and Ginn
22 2014).

23 According to the intrinsic characteristics of hypersaline environments (high biological
24 productivity and low biodiversity), resource limitation is less likely to inhibit an invasion or colonization,
25 and species richness should have little influence on invasion success (Hobbs and Huenneke 1992; Davis
26 et al. 2000; Kennedy et al. 2002). Disturbance in these systems usually disrupts the behavior of
27 individuals, reducing population fitness and raising the probabilities of success for invading colonists
28 (Davis et al. 2000). The American *A. franciscana* is an interesting case of expanding species exhibiting a
29 high genetic diversity, part of which is correlated with key fitness traits, contributing to its invasibility
30 capabilities (Gajardo and Beardmore 1989; Pilla and Beardmore 1994; Kappas 2001). Increased genetic
31 diversity was found in many other introduced species (Vidal et al. 2010), resulting from a combination of
32 multiple local introductions of several, or the same, alien origin (Kolbe et al. 2008), that led to numerous
33 translocations from the sites of introduction. This genetic diversity, broadly displayed by the females
34 from the original Great Salt Lake population (Gajardo and Beardmore 1989) provides adaptive responses
35 to variable environmental conditions (Gajardo et al. 2002). Among these, the presence of disturbing
36 xenobiotics including chlorpyrifos should not be disregarded. It could be the basis of an important
37 adaptation into its original range, as well as into the invaded areas (i.e. here in “*La Trinitat*” salterns
38 years after its arrival). Information dealing with the presence of xenobiotics in continental American
39 basins unveils the presence of diverse chlorpyrifos levels in streams draining agricultural settings and

1 urban areas to important hydrological basins, levels that sometimes exceed criteria established for the
2 protection of aquatic life (Larson et al. 1999).

3 This may not be the case for the native diploid *A. parthenogenetica* populations. Geographically
4 more widely distributed than their sexual relatives, their success relies partially on the existence of a
5 controversial general purpose genotype (GPG) (Van Doninck et al. 2002), able to compensate the low
6 genetic plasticity in parthenogenetic lineages. This genotype probably explains the flourishing asexual
7 organisms in certain environments, based in reproductive efficiency, faithful replication of this GPG, and
8 the generation of specialized genotypes, contrasting with the restricted geographic distribution and strong
9 genetic structure of sexual *Artemia* species. But, despite the broad expansion of parthenogens, possibly
10 facilitated by an increased availability of suitable habitats provided by anthropogenic activities (Muñoz
11 and Pacios 2010), the absence of mechanisms for rapid genetic change, that has earned asexuals the label
12 of evolutionary dead ends (Butlin, 2002), is on the basis of their limitation in the genetic plasticity
13 necessary to resist in disturbed environments, especially those polluted with new xenobiotics.

14 15 **5. Conclusion**

16
17 The results of this study evidence that both AF and PD showed an elevated tolerance to high
18 ranges of chlorpyrifos, but AF survived better and its fecundity was less affected by the exposure to the
19 pesticide than PD. The higher fecundity of AF is a selective advantage in colonization processes leading
20 to its establishment as NIS. Besides, under the potential selective pressure of abiotic factors, such as the
21 presence of toxicants, its higher resistance in terms of survival and biological fitness also indicates out-
22 competitive advantages.

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25
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1 **Figure legends**

2
3 **Fig 1.** Survival curves of *A. franciscana* and *A. parthenogenetica* exposed to different concentrations of
4 chlorpyrifos during life table experiments. (*) *A. parthenogenetica* individuals exposed to 5 µg/l of
5 chlorpyrifos reached 100% mortality after 7 days (data not shown).
6

7 **Fig 2.** Principal Component Analysis (PCA) of the life table variables of *A. franciscana* and *A.*
8 *parthenogenetica* exposed at different concentrations of chlorpyrifos. (A) Component plot. (B) Score
9 plot: cases identified by species. (C) Score plot: cases identified by concentration of toxicant. For
10 abbreviations see the text.
11

12 **Fig 3.** *A. franciscana* exposed to different concentrations of chlorpyrifos. (A) Effects of treatment on age-
13 specific fertility measured relative to the control (C) (left), and contributions of these effects to the impact
14 of chlorpyrifos on population growth rate (right). (B) Effects of treatment on age-specific survival
15 probabilities measured relative to the control (C) (left), and contributions of these effects to the impact of
16 chlorpyrifos on population growth rate (right).
17

18 **Fig 4.** *A. parthenogenetica* exposed to different concentrations of chlorpyrifos.(A) Effects of treatment on
19 age-specific fertility measured relative to the control (C) (left), and contributions of these effects to the
20 impact of chlorpyrifos on population growth rate (right). (B) Effects of chlorpyrifos treatment on age-
21 specific survival probabilities measured relative to the control (C) (left), and contributions of these effects
22 to the impact of chlorpyrifos on population growth rate (right).
23

24 **Fig 5.** Cholinesterase (ChE) activity in *A. franciscana* at different developmental stages after chronic
25 exposure to different concentrations of chlorpyrifos. Values are given as means ± SD (standard
26 deviation); N= 10-18. (A) Females and (B) males. (*) Denotes significant differences from control (C)
27 (Dunnet´s test, P < 0.05).
28

29 **Fig 6.** Cholinesterase (ChE) activity in *A. parthenogenetica* at different developmental stages after
30 chronic exposure to different nominal concentrations of chlorpyrifos. Values are given as means ± SD
31 (standard deviation); N =10-16 individuals. (*) Denotes significant differences from control (C)
32 (Dunnet´s test, P < 0.05).
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34 **Fig 7.** Cholinesterase (ChE) activity in non-exposed (C: control group) individuals of *A. franciscana* and
35 *A. parthenogenetica* at different developmental stages. Values are given means ± SD (standard
36 deviation); N = 10-18 individuals. Mn: metanauplii, JM: juvenile male, JF: juvenile female, AM: adult
37 male, AF: adult female, J: juvenile, A: adult. (*) Denotes significant differences (Tukey´s test, P < 0.05).
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Table 1 Reproductive parameters obtained from life table experiments for *A. franciscana* (AF) exposed to different concentration of chlorpyrifos. Mean \pm SD (standard deviation). See text for more details.

Reproductive parameters	Chlorpyrifos concentrations				
	C	CA	0.1 $\mu\text{g/l}$	1 $\mu\text{g/l}$	5 $\mu\text{g/l}$
Temporal parameters					
PRP (days)	19.63 \pm 2.03	19.80 \pm 1.06	19.39 \pm 1.79	18.05 \pm 2.37	19.11 \pm 1.88
P/B (days)	5.12 \pm 0.48	4.83 \pm 0.48	4.87 \pm 0.38	4.71 \pm 0.42	4.73 \pm 0.87
Fecundity					
B	7.32 \pm 1.63 ^{ab}	6.25 \pm 1.89 ^b	7.56 \pm 2.23 ^{ab}	8.58 \pm 1.35 ^a	6.05 \pm 2.01 ^b
OFF	1194.89 \pm 331.11 ^{ab}	999.00 \pm 378.36 ^b	1227.72 \pm 449.97 ^{ab}	1410.58 \pm 337.95 ^a	1031.21 \pm 448.08 ^b
OFF/B	162.03 \pm 34.67	158.38 \pm 30.82	162.45 \pm 30.19	163.69 \pm 26.24	165.49 \pm 38.25
Type of reproduction					
OVO (%)	38.35 \pm 28.02	27.94 \pm 32.60	26.54 \pm 35.54	34.53 \pm 35.97	43.31 \pm 31.94
OVIP (%)	61.65 \pm 28.02	72.06 \pm 32.60	73.46 \pm 35.54	65.47 \pm 35.97	56.69 \pm 31.94
Quality of reproduction					
VN (%)	95.81 \pm 9.64	95.25 \pm 7.06	98.42 \pm 1.33	88.79 \pm 27.74	80.30 \pm 28.27
NVN (%)	4.19 \pm 9.64	4.75 \pm 7.06	1.42 \pm 1.35	11.15 \pm 27.76	19.70 \pm 28.27
VC (%)	99.09 \pm 2.07	98.37 \pm 5.31	95.17 \pm 8.17	88.84 \pm 12.38 [*]	79.66 \pm 30.18
NVC (%)	0.91 \pm 2.07	1.63 \pm 5.31	4.83 \pm 8.17	11.16 \pm 12.38 [*]	20.34 \pm 30.18

Different letters within the same row denotes significant differences (*post hoc* Turkey's test, $p < 0.05$)

(*) Indicates significant differences from C (control group) (Games-Howell's test, $p < 0.05$)

PRP: pre-reproductive period; P/B: period between broods; B: total number of broods per female; OFF: total offspring per female; OFF/B: total offspring per brood; OVO: ovoviviparous (nauplii); OVIP: cysts; VN: viable nauplii; NVN: dead plus abortive embryos; VC: viable cysts; NVC: non-viable cysts.

Table 2 Reproductive parameters obtained from life table experiments for *A. parthenogenetica* (PD) exposed to different concentration of chlorpyrifos. Mean \pm SD (standard deviation). See text for more details.

Reproductive parameters	Chlorpyrifos concentrations				
	C	CA	0.1 $\mu\text{g/l}$	1 $\mu\text{g/l}$	5 $\mu\text{g/l}$
Temporal parameters					
PRP (days)	31.93 \pm 4.29	32.38 \pm 3.99	32.06 \pm 3.92	29.56 \pm 3.0	-
P/B (days)	5.94 \pm 0.53 ^a	5.74 \pm 0.31 ^{ab}	6.02 \pm 0.72 ^a	5.19 \pm 0.45 ^b	-
Fecundity					
B	4.71 \pm 1.49 ^{ab}	5.08 \pm 1.55 ^a	3.69 \pm 0.70 ^b	3.44 \pm 0.53 ^c	-
OFF	584.93 \pm 363.99	587.08 \pm 242.80	394.44 \pm 141.53	258.11 \pm 83.94 [*]	-
OFF/B	117.72 \pm 49.44	113.46 \pm 30.36	106.0 \pm 28.61	74.07 \pm 16.40 ^{**}	-
Type of reproduction					
OVO (%)	17.02 \pm 27.84	20.39 \pm 16.18	7.38 \pm 15.08	44.72 \pm 34.30	-
OVIP (%)	82.98 \pm 27.84	79.61 \pm 16.18	92.62 \pm 15.08	55.28 \pm 34.30	-
Quality of reproduction					
VN (%)	62.70 \pm 36.74	63.40 \pm 29.18	68.63 \pm 34.68	71.65 \pm 20.09	-
NVN (%)	37.30 \pm 36.38	36.60 \pm 29.18	31.37 \pm 34.68	28.35 \pm 20.09	-
VC (%)	78.79 \pm 24.20	84.88 \pm 19.33	92.45 \pm 10.98	91.09 \pm 14.01	-
NVC (%)	21.21 \pm 24.20	15.12 \pm 19.33	7.55 \pm 10.98	8.91 \pm 14.01	-

Different letters within the same row denotes significant differences (*post hoc* Turkey's test, $p < 0.05$)

(*) Indicates significant differences from all groups (Games-Howell's test, $p < 0.05$)

(**) Indicates significant differences from C (Games-Howell's test, $p < 0.05$)

PRP: pre-reproductive period; P/B: period between broods; B: total number of broods per female; OFF: total offspring per female; OFF/B: total offspring per brood; OVO: ovoviviparous (nauplii); OVIP: cysts; VN: viable nauplii; NVN: dead plus abortive embryos; VC: viable cysts; NVC: non-viable cysts

Table 3 Population growth rate (λ) in *A. franciscana* (AF) and *A. parthenogenetica* (PD) populations for the different treatments assayed. Values in brackets indicate percentage deviation from control (C).

Species	Chlorpyrifos concentrations				
	C	CA	0.1 $\mu\text{g/l}$	1 $\mu\text{g/l}$	5 $\mu\text{g/l}$
AF	2.72	2.44 (-10.3)	2.49 (-8.5)	2.41 (-11.4)	2.35 (-13.6)
PD	1.88	1.83 (-2.6)	1.59 (-15.4)	1.45 (-22.9)	-

Fig.1

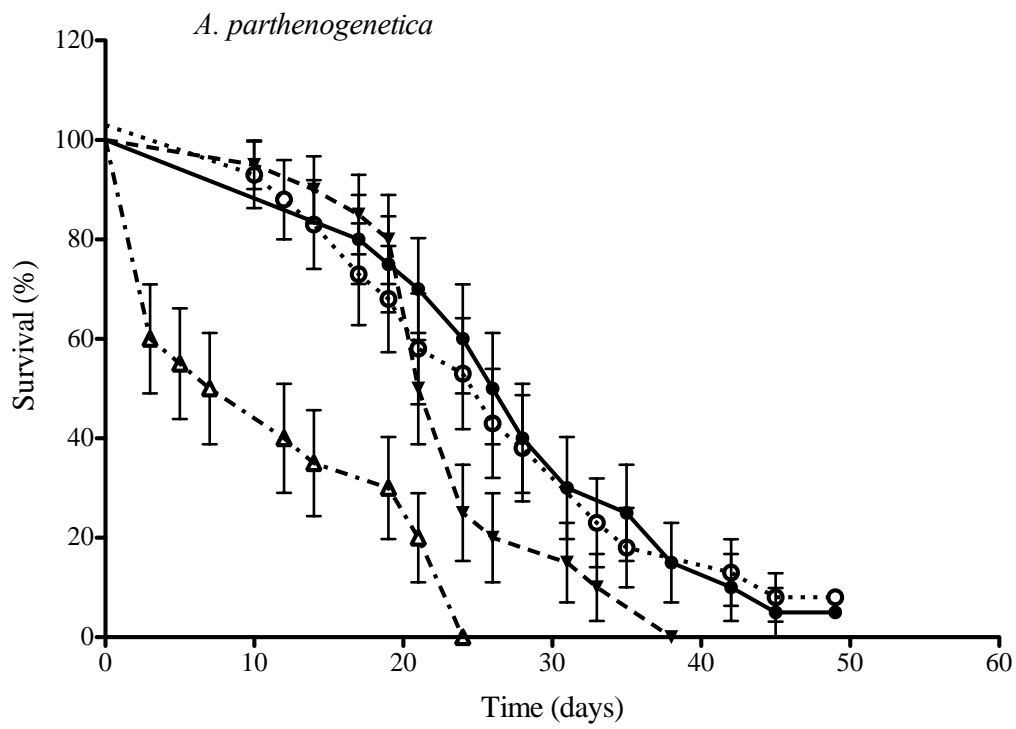
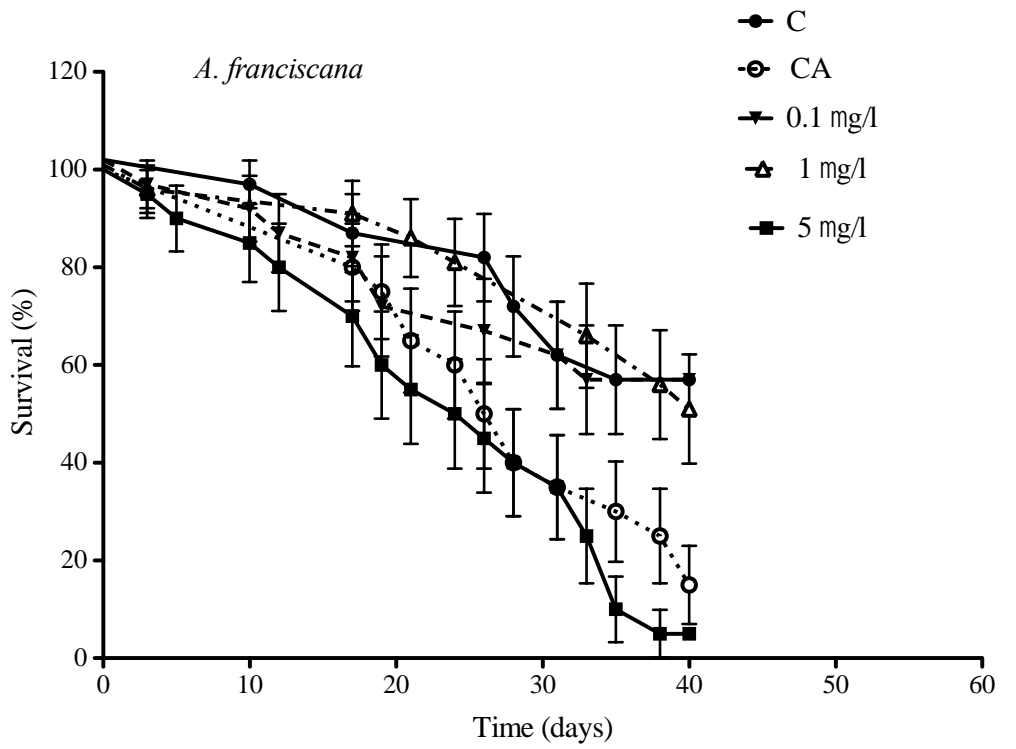
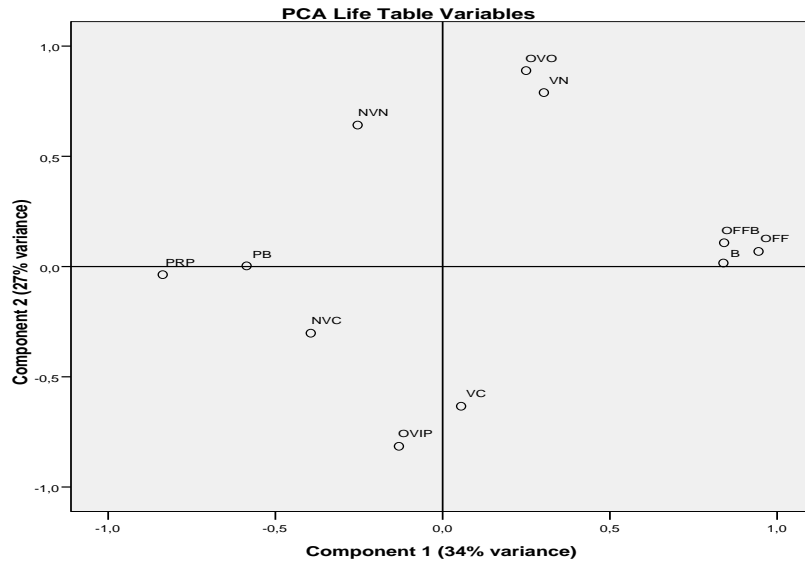
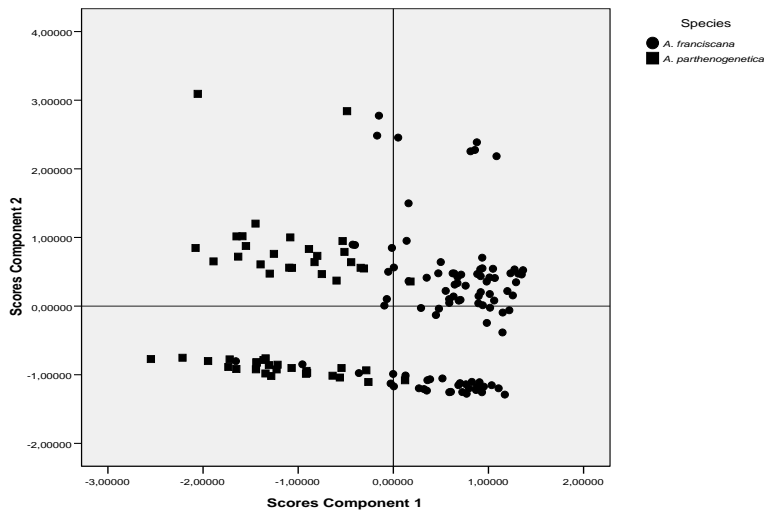


Fig 2

A



B



C

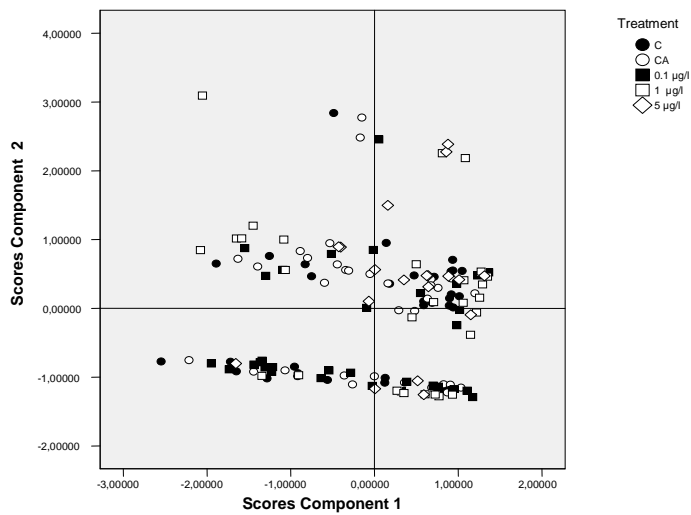
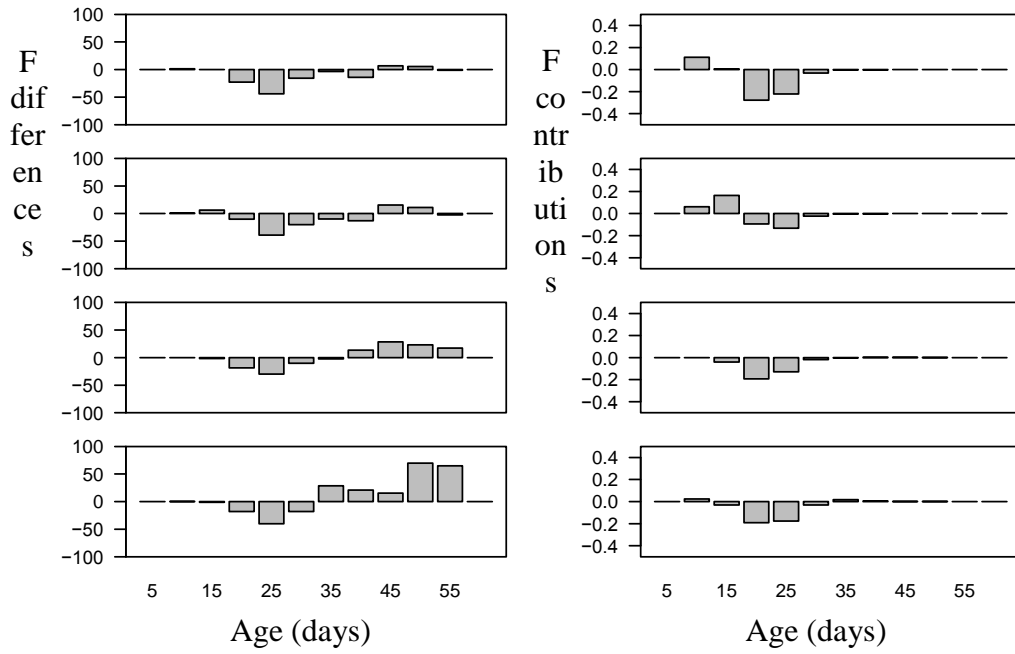


Fig 3

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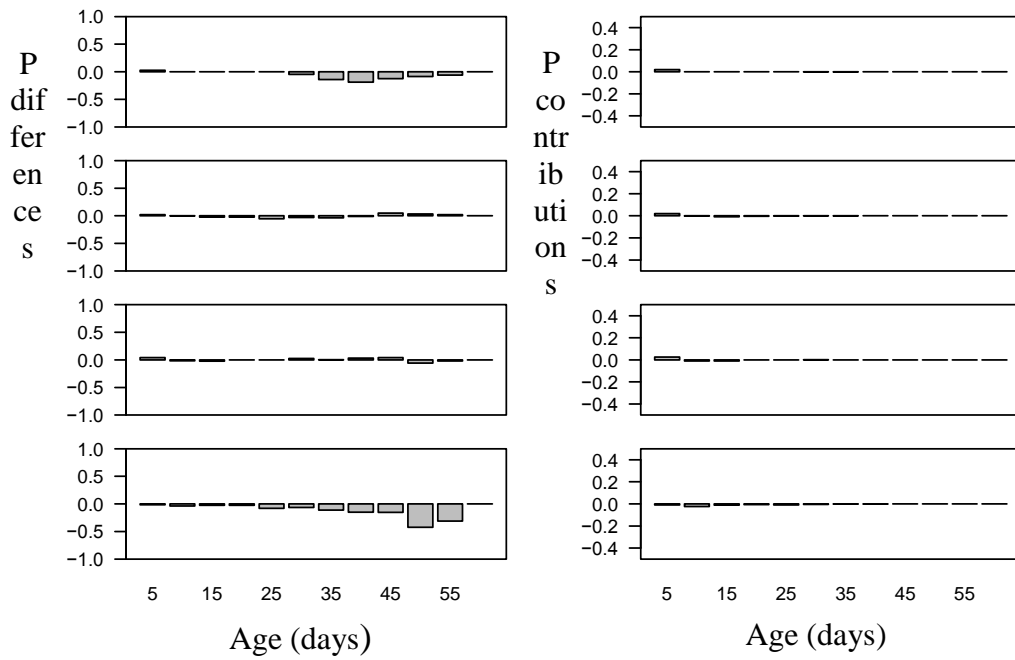
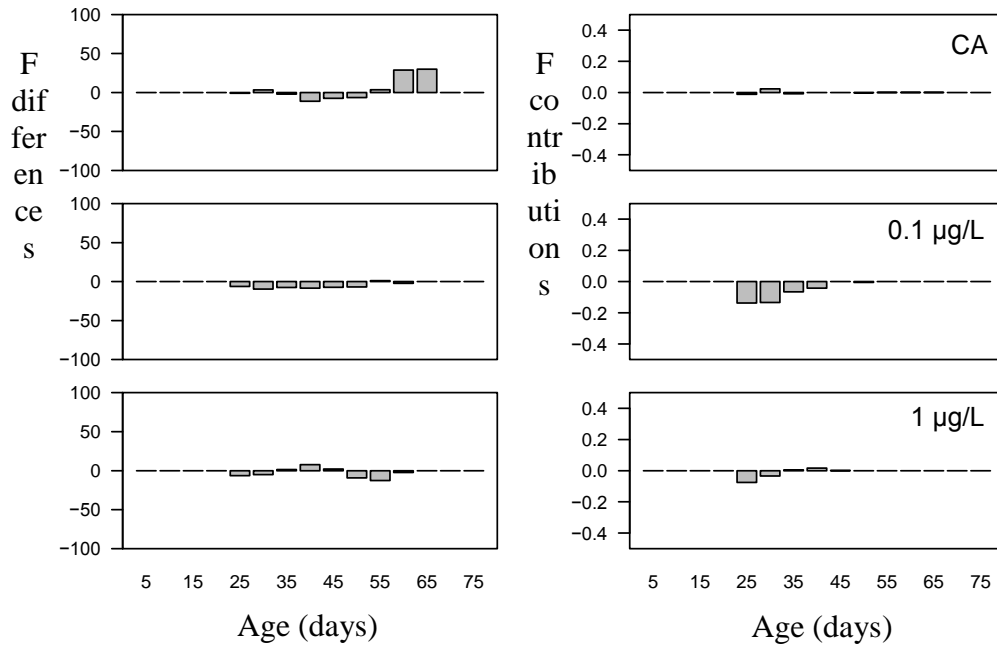


Fig.4

A



B

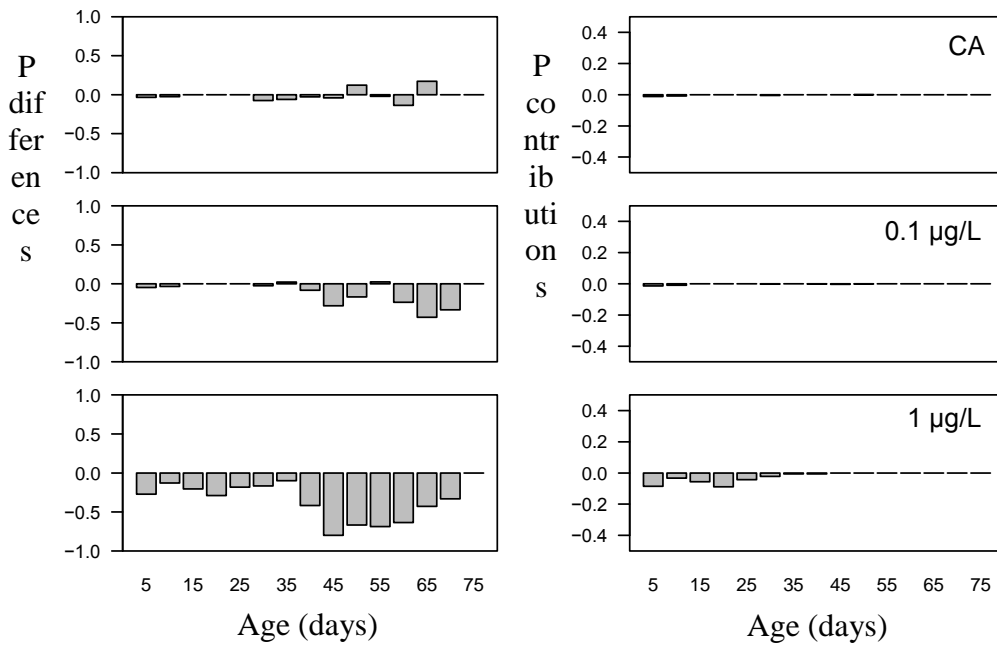


Fig 5

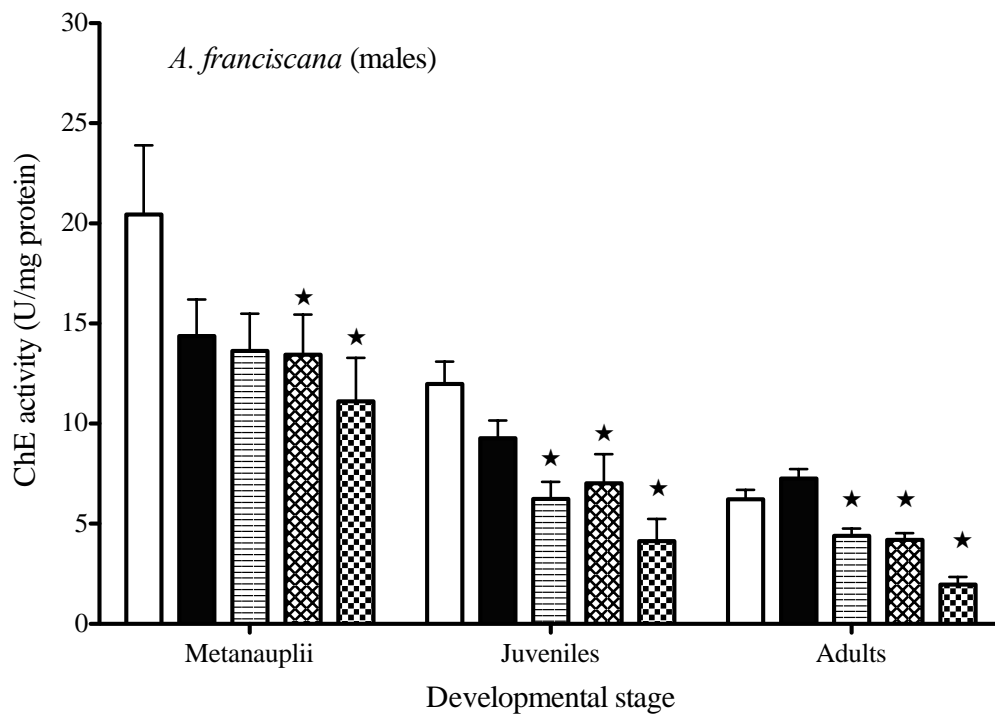
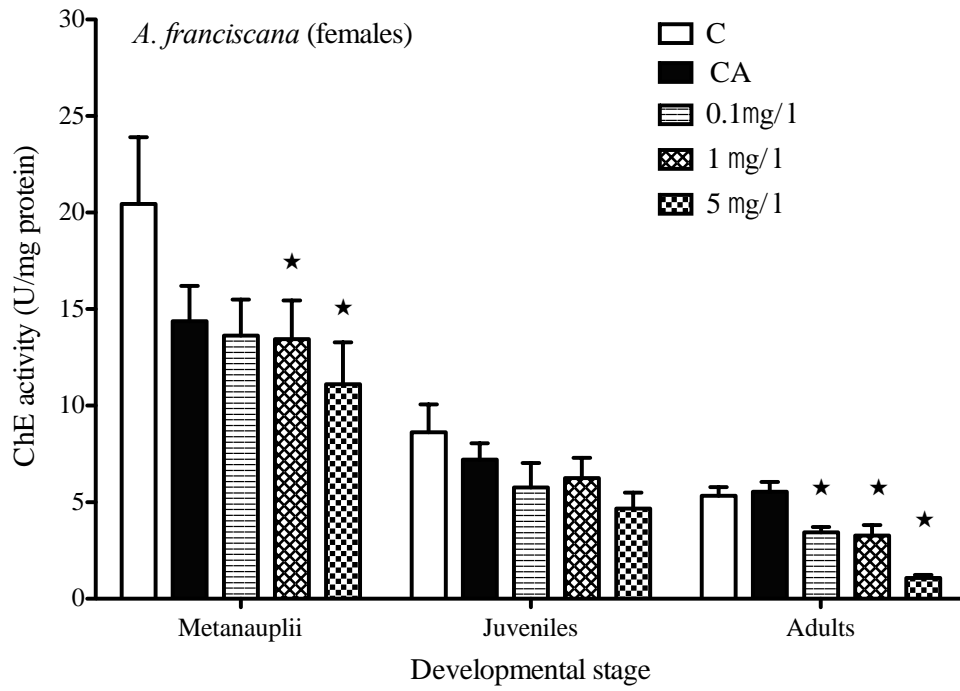


Fig 6

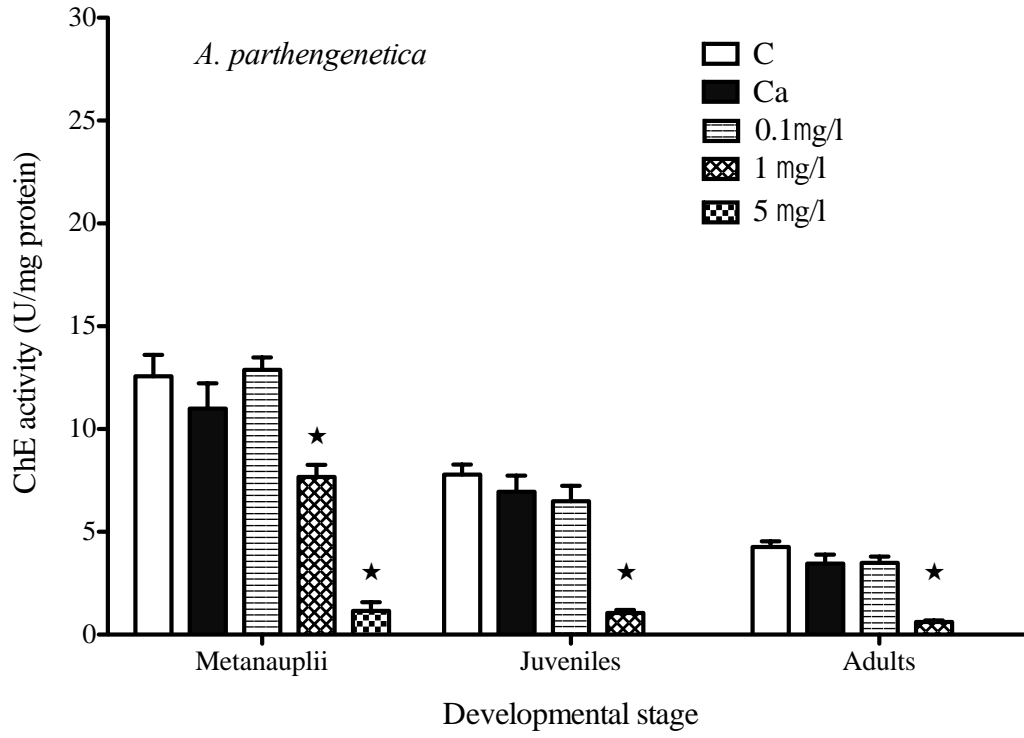
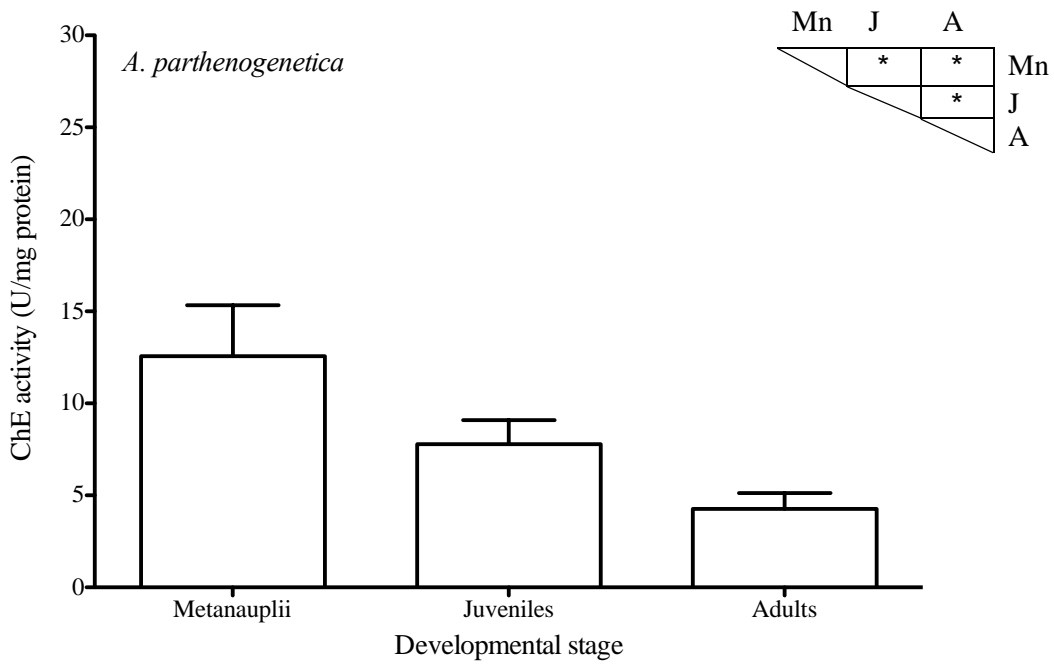
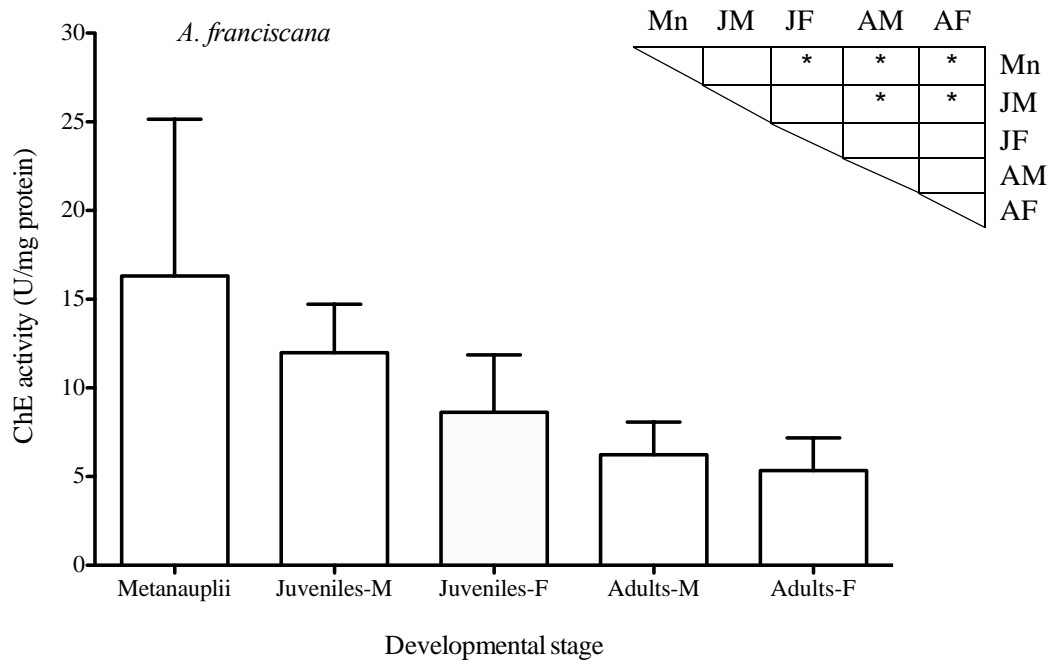


Fig 7



- *Artemia* species display an elevated tolerance to high ranges of chlorpyrifos.
- *A. franciscana* survived better and its fecundity was less affected by chlorpyrifos.
- The higher fecundity of *A. franciscana* is a selective advantage in colonization processes.
- Higher survival and biological fitness in *A. franciscana* indicate out-competitive advantages.