



ORIGINAL ARTICLE

Seasonal occurrence and hatching success of benthic eggs of calanoid copepods in the Bahía Blanca Estuary, Argentina

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Abstract

The seasonal abundance and hatching success of calanoid benthic eggs were examined during the coexistence of *Acartia tonsa* and *Eurytemora americana* in the Bahía Blanca Estuary (BBE). The dormancy behaviour of eggs extracted from surface sediment layers was tested by simulating seasonal conditions during incubation. There was a greater abundance of benthic eggs of *E. americana* ($\leq 8.68 \times 10^6$ eggs m^{-2}) than of *A. tonsa*, the latter consistently showing low egg abundance ($\leq 0.8 \times 10^6$ eggs m^{-2}). The emergence of most *E. americana* nauplii occurred at 17°C after the experimental warming phase (refractory phase of 280 days), confirming the diapause character of these eggs. The emergence of the first *A. tonsa* nauplii from initial cold season samples occurred at 9–12°C after 60–70 days of incubation. However, a 180-day delay in hatching at temperatures $\geq 15^\circ C$ was also observed in *A. tonsa* eggs from the last sampling days of the cold season, thus suggesting an intermediate behaviour between diapause and quiescence. The benthic egg bank in the inner zone of the BBE provides a temporary reservoir for both key copepods, thus contributing in their survival.

Key words: Resting calanoid eggs, diapause eggs, *Eurytemora americana*, *Acartia tonsa*, benthic egg bank, dormancy strategy

Introduction

Resting eggs are considered crucial to the life cycle of copepods, whose re-establishment and survival in subsequent years depend on them (Giangrande et al. 1994; Marcus & Boero 1998). Resting copepod eggs can develop different types of dormancy, ranging from quiescence to diapause (Grice & Marcus 1981; Mauchline 1998). Quiescence is a response of subitaneous (ready-to-hatch) eggs to adverse environmental conditions. In contrast, diapause is a state of genetically controlled arrested development and requires a prior refractory phase (Ban 1992; Giangrande et al. 1994; Belmonte & Pati 2007). Resting eggs may accumulate throughout the year in the bottom sediment, forming benthic egg banks, and remain viable in a dormant state (Uye 1985; Marcus 1996; Marcus & Boero 1998; Jiang et al. 2004). Egg banks are reservoirs of genetic and ecological information about planktonic populations (Hairston &

De Stasio 1988; Ellner & Hairston 1994; Limburg & Weider 2002; Avery 2005) and have been detected in sediments of several coastal ecosystems with egg density values ranging from 10^4 to 10^7 egg m^{-2} (Kasahara et al. 1975; Belmonte et al. 1995; Uriarte & Villate 2006; Glippa et al. 2011).

In the Bahía Blanca Estuary (BBE), the existence of benthic egg banks has been documented and diapause eggs of the rotifer *Synchaeta* sp. and eggs of copepod species have been found (Diodato et al. 2006). Although other species of calanoid copepods (e.g. *Paracalanus parvus* (Claus, 1863), *Labidocera fluvialis* Dahl, 1894, *Calanoides carinatus*) (Kröyer, 1849) are found in the plankton community of the BBE (Hoffmeyer 2004; Hoffmeyer et al. 2008), only eggs of *Acartia tonsa* Dana, 1849 and *Eurytemora americana* Williams, 1906 have been observed so far in the sediments (Diodato et al. 2006). These two copepod species dominate the mesozooplankton community in the inner zone of the BBE and are

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considered key species in the trophic web of this estuary (Hoffmeyer et al. 2009; Lopez-Cazorla et al. 2011). *Eurytemora americana* was introduced via ballast water in the mid-1980s, causing changes in the mesozooplankton structure of the estuary (Hoffmeyer 1994, 2004). This species has increased in density in the years since its arrival, coexisting with *A. tonsa* from June to October and producing a slight exclusion of this copepod in the inner zone (Hoffmeyer et al. 2008). This invader copepod emerges only during the cold austral season and early spring (Hoffmeyer et al. 2009; Berasategui et al. 2009a, 2009b, 2012), whereas *A. tonsa* occurs throughout the year, displaying minimum abundance during the cold season (Sabatini 1989). It was suggested that *A. tonsa* produces resting eggs in the estuary during this colder season (Sabatini 1989; Hoffmeyer et al. 2009). Although the production of diapause eggs has been reported for *A. tonsa* in other estuaries (Zillioux & Gonzalez 1972; Grice & Marcus 1981; Marcus 1996; Castro-Longoria 2001), the high hatching rate at 18°C of eggs produced during the cold season suggests that *A. tonsa* does not display diapause in the BBE (Berasategui et al. 2012; AAB, pers. obs.). These benthic eggs are important agents of local recolonization after adverse environmental conditions (Hoffmeyer et al. 2009), but there are no studies evaluating their hatching success or dormancy type.

Recent studies in the BBE show that *E. americana* manifests two markedly distinct types of reproductive behaviour, depending on the prevailing environmental conditions (Berasategui et al. 2009a, 2009b). Results of experimental incubations of field-collected females and electron microscopy observations (TEM & SEM) (Berasategui et al. 2012) revealed the existence of significant differences in egg size and different chorion appearance through the *E. americana* population pulse. The eggs produced after the population peak exhibited a sponge-like appearance (95.78–99.33 µm diameter) and were identified as diapause eggs based on their inability to hatch even after 3 months of incubation under favourable conditions. It was suggested that these resting eggs may not have hatched because they required a refractory phase in order for development of the embryo to continue (Berasategui et al. 2012). However, no studies so far have reported a dormancy strategy for *E. americana* benthic eggs which would confirm the existence of a refractory phase. Moreover, it has been suggested that the first *E. americana* nauplii at the beginning of the population pulse in the BBE are recruited from benthic resting eggs in June, when the austral cold season starts (Diodato et al. 2006; Hoffmeyer et al. 2008). This first appearance of nauplii has been associated with

low surface temperatures (6–7.2°C), low daily irradiation (5 MJ m⁻²) and the highest phytoplanktonic biomass values in the water column (Hoffmeyer et al. 2009).

Because benthic eggs are presumably crucial to *A. tonsa* and *E. americana* population dynamics and there are very few studies focusing on their dormancy strategy, the aims of the present study were to (i) describe the seasonal occurrence of benthic eggs of both copepod species during the coexistence period and (ii) characterize the hatching success of resting eggs of both species from benthic banks of the Bahía Blanca Estuary.

Materials and methods

Sampling procedures

Sampling and measurements were conducted at Cuatros Port during the cold austral season 2007, with an approximately fortnightly frequency (12 June, 29 June, 17 July, 1 August, 15 August, 5 September, 20 September, 2 October, 8 October), covering the entire *Eurytemora americana* annual pulse and the period of adverse conditions up to *Acartia tonsa* population development. Cuatros Port is a turbid, shallow harbour (mean depth of 6 m) located in the innermost zone of the BBE (38°44'49"S–62°23'12"W) (Figure 1). It is a vertically mixed estuary with a relative homogeneity of physico-chemical conditions in the water column, mainly in terms of temperature, pH and turbidity (Freije et al. 2008). The high turbidity in the estuary, with values ranging between 50 and 300 NTU, is the combined result of winds and tidal currents provoking the resuspension of fine sand and silt–clay sediments (Perillo & Piccolo 1999). The sedimentation rate of suspended particulate matter ranges from 75 to 221 mg m⁻² day⁻¹ during the winter (Guinder et al. 2012), the main factors contributing to diminished transparency in the water column being plankton and detritus (Guinder et al. 2009). There are marked seasonal variations in temperature (5.1–26.4°C) and salinity (17.9–41.3 Practical Salinity Scale) and hypersaline conditions are usually recorded during hot dry summers (Freije et al. 2008). The average concentration of dissolved oxygen (DO) was close to 7 mg l⁻¹, reaching 13 mg l⁻¹ during the periods of highest phytoplankton production in winter and late summer (Popovich & Marcovecchio 2008).

Mesozooplankton samples were taken by means of horizontal hauls near the surface layer during ebb tide, using a 0.30-m diameter open-mouth, 200-µm mesh net containing a General Oceanic® mechanical flowmeter. One sample was collected on each sampling date and was fixed in 4% buffered formalin. The

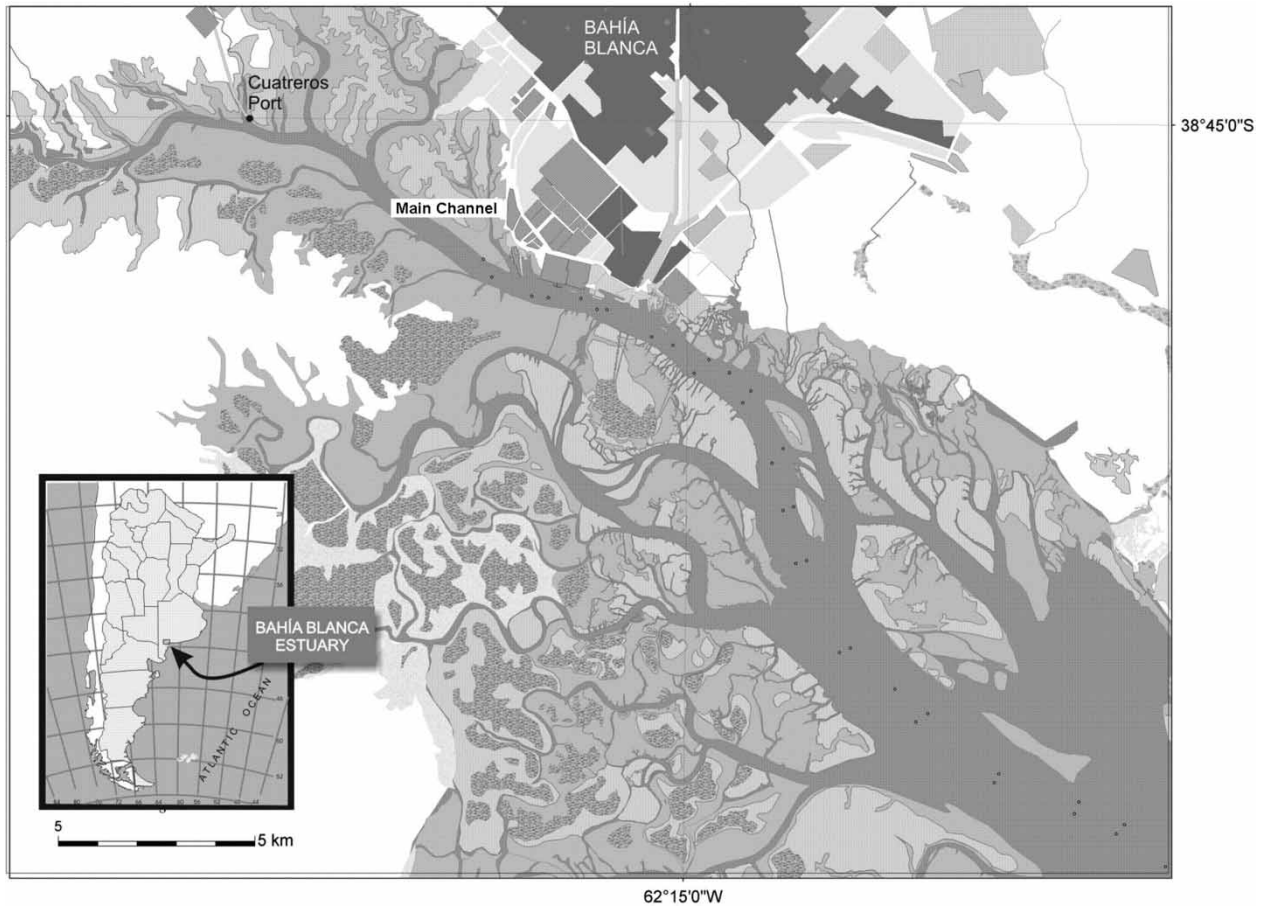


Figure 1. Map of the Bahía Blanca Estuary (BBE), Argentina, showing the location of the sampling station (Cuatreros Port).

abundance of each species stage was calculated according to Boltovskoy (1981) and expressed as ind. m^{-3} . Copepodites I to V, females and males of *E. americana* and *A. tonsa* were identified according to Grice (1970), Heron (1964), Landry (1983) and Sabatini (1990). On each sampling date, surface sediment samples ($n=4$) were collected using an Eckman dredge. Sediment samples were taken from the top centimetre of the bank surface using a spatula and circular 6.5 cm diameter moulds and then placed in 250 cm^3 plastic containers. The samples were preserved at 4–6°C under hypoxic conditions (dissolved oxygen levels $\leq 2 \text{ mg l}^{-1}$) for a storage period shorter than one week to prevent microbial activity until laboratory processing (Peck & Holste 2006). Additional surface water samples (0.5–1 m depth) were taken for experimental laboratory incubations (10–20 litres on each sampling date). Surface water temperature, salinity and DO were recorded using a HORIBA® multiparameter probe.

Benthic eggs and experimental incubation

In order to calculate the total average abundance of benthic calanoid eggs per area (BEA) on each

sampling date, two samples of surface sediment were used. To isolate all calanoid copepod eggs from each sample, the sediment was passed through a series of 180- and 53- μm sieves and washed with filtered seawater ($\leq 30 \mu\text{m}$). The eggs and fine sediment particles retained by the 53- μm sieve were concentrated in 100 ml of seawater. This volume was fractionated into 1 ml aliquots and placed in a Bogorov chamber to facilitate identification and quantification of eggs. Each aliquot was then examined under a stereo microscope with the light source placed under the sample and maximum magnification ($40\times$). This procedure was repeated several times until the entire sample was analysed. The identification of eggs was carried out by means of differences in the varying light intensities and on the basis of their morphological description under optical microscopy (OM) (Diodato et al. 2006; Berasategui et al. 2012). After identifying and counting the benthic eggs, the contribution of each species to BEA was calculated (BEAEa, average abundance of benthic eggs of *Eurytemora americana* and BEAAt, average abundance of benthic eggs of *Acartia tonsa*).

In order to assess hatching success under simulated seasonal variations, benthic calanoid eggs were

Table I. Experimental design to evaluate the hatching success of benthic eggs. Conditions during the previous acclimation period, temperature *in situ* ± 1 (T, °C), salinity measured in practical salinity units (S), and dissolved oxygen (DO, mg l⁻¹). The following three experimental incubation phases are shown on the right.

Benthic eggs Sampling date	Acclimation period - <i>in situ</i> conditions					
	T	S	DO			
12 Jun.	8.00	33.75	11.45	Springtime	Warming	Autumn-wintertime
29 Jun.	6.70	33.94	10.80			
17 Jul.	5.25	34.10	10.03			
01 Jul.	5.70	32.98	7.80			
15 Aug.	7.00	32.92	7.28			
05 Sep.	11.90	37.51	7.01			
20 Sep.	12.00	27.63	7.50			
02 Oct.	14.20	29.86	8.60			
08 Oct.	15.80	28.58	9.20			

isolated as mentioned above from the remaining sediment samples. All eggs found on each sampling date (N°BEI) were placed in plastic dishes filled with 500 ml filtered seawater ($\leq 30 \mu\text{m}$). Each set of benthic eggs was first exposed to an acclimation period under *in situ* natural conditions for 15–20 days. Unhatched eggs of this first period (N°BEII) were chronologically exposed to three different incubation phases: springtime, warming phase and autumn–wintertime (Table I). Experimental conditions of these incubation phases corresponded to environmental conditions in the study area from September to January 2007 and 2008 (springtime and warming phase) and April to August 2006 and 2007 (autumn–wintertime). The springtime phase comprised raising temperatures (5, 12, 14.2 and $15.8^\circ\text{C} \pm 0.5^\circ\text{C}$) and changes in salinity (27.6, 27.6, 29.9 and 28.6), in four time-steps every 60 days approximately, whereas the warming phase consisted of increasing temperatures (18, 19 and $20 \pm 0.5^\circ\text{C}$) and salinities (33, 34 and 35.5) in three time-steps every 30 days and a decrease in DO values up to a minimum of 4 mg l^{-1} , simulating values recorded in the water column during the warm season (Freije et al. 2008). The autumn–wintertime phase consisted of decreases in temperature (17.5, 14.0 and $9 \pm 0.5^\circ\text{C}$), increases in salinity (30.5, 32.5 and 33.5) and *in situ* DO values ($> 8 \text{ mg l}^{-1}$), the changes being made in three time-steps at approximately 20 days. The incubations were performed in a temperature-controlled culture room, a thermostatic bath and refrigerated chamber with 10 h of photoperiod at low light intensity (5.5 W m^{-2}). This photoperiod was applied because it corresponded to a high recruitment of nauplii in the field (Hoffmeyer et al. 2009). Salinity, temperature and DO were checked every 7 days using a Hanna® digital multisensor probe. Oxygen concentration was regulated by an aerator and salinity concentration was modified by the addition of distilled water. Incubation water was

renewed with *in situ* seawater ($\leq 30 \mu\text{m}$) every 7 days by soft reverse filtration of the first three-quarters of the incubation volume, keeping the emerging nauplii in a $60\text{-}\mu\text{m}$ sieve. These nauplii were incubated again under the same hatching conditions of temperature and salinity, for 15–20 days with seston $\leq 60 \mu\text{m}$ and a mixture (1:2) of *Isochrysis galbana* and *Tetraselmis suecica* to promote their development and facilitate their taxonomic identification. Nauplii and other development stages accumulated were then fixed in 4% formaldehyde seawater solution. The total quantification of nauplii–copepodites and unhatched eggs was carried out using OM and a stereo microscope at the end of the experimental time for each temperature. The development stages were identified by OM following the taxonomic description of Grice (1970) and Landry (1983). Cumulative average hatching for each species (% HBEEa, *E. americana* and % HBEAt, *A. tonsa*) at each experimental temperature during each incubation phase was expressed as a percentage of the initial number of total benthic eggs (N°BEI and N°BEII). Unhatched eggs were treated using neutral red staining at the end of incubation to assess their viability (Dressel et al. 1972; Hoffmeyer et al. 2005).

Results

Temporal variation in environmental conditions and population abundance

High salinity values above 30 were registered from 12 June to 5 September (Figure 2). A decrease in salinity was recorded after 5 September, reaching values near 27. Higher values of dissolved oxygen ($10\text{--}11 \text{ mg l}^{-1}$) were recorded on the first three sampling dates, showing a decline on 1 August. Low temperatures were recorded ($5\text{--}7^\circ\text{C}$) from 12 June to 15 August, showing a minimum value of 5.25°C

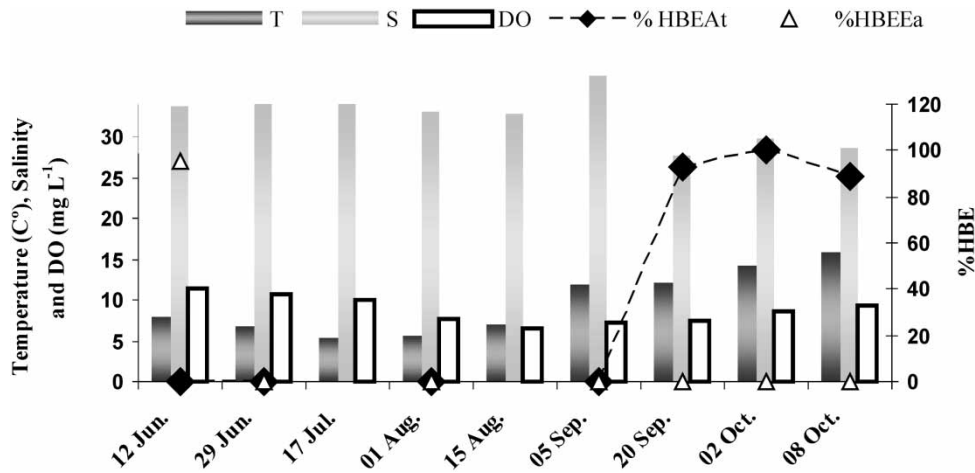


Figure 2. Incubations of benthic eggs. Acclimation period under *in situ* natural conditions. Temperature (T); salinity (S); dissolved oxygen (DO); cumulative average hatching success of *Eurytemora americana* (% HBEEa) and cumulative average hatching success of *Acartia tonsa* (% HBEAt).

on 17 July. The temperature increased (≥ 11 °C) on 5 September.

The highest abundance of *Eurytemora americana* copepodites was recorded during the population pulse (5 September), made up mainly of copepodites I to III (Figure 3A). Female abundance was slightly higher (10 ind. m^{-3}) from the end of June to 5 September and male abundance was higher than female abundance (19 ind. m^{-3} as opposed to 5 ind. m^{-3}) at the end of the pulse. A low abundance of *Acartia tonsa* was observed during the whole study period, with an increase in abundance of copepodites after September (Figure 3B). The lowest *A. tonsa* abundance of all stages (< 20 ind. m^{-3}) coincided with the lowest temperatures (5.25–6.70 °C) and the highest salinities (32.73–36.62) recorded *in situ*. Males and females were detected in very low abundances (≤ 2 ind. m^{-3}) from 5 to 20 September. It is likely that the population abundances of both copepod species were underestimated in the present study owing to the use of a 200- μm mesh size net that fails to capture naupliar and even first copepodite stages.

Benthic eggs and experimental incubations

Very low abundances of benthic calanoid eggs were observed ($< 0.5 \times 10^6$ eggs m^{-2}) from 12 June to 5 September (Figure 3C). Benthic eggs of *Eurytemora americana* were not detected in samples from 29 June to 15 August. After 5 September (*E. americana* population peak) an increase in BEAEa was observed, showing the highest abundance (7.89×10^6 eggs m^{-2}) on 2 October; the eggs were found mostly in groups, as incomplete egg clutches. Low abundances of *Acartia tonsa* benthic eggs were observed during the study period (BEAAt $\leq 0.8 \times 10^6$ eggs

m^{-2} , Figure 3C) and no eggs were found in the samples of 12 June, 17 July and 15 August.

Experimental incubations could only be performed on seven dates since no calanoid eggs were found in benthic samples from 17 July and 15 August (N^oBEI: 12 June = 35Ea and 0At, 29 June = 0 Ea and 28 At, 1 August = 0 Ea and 34 At, 5 September = 38 Ea and 34 At, 20 September = 177 Ea and 42 At, 2 October = 1047 Ea and 105 At, 8 October = 154 Ea and 24 At). During the acclimation period, a high hatching success of benthic eggs (%HBE) was observed only on the first and last sampling dates, corresponding to *E. americana* and *A. tonsa*, respectively (Figure 2). Unhatched eggs found during this first acclimation period were exposed to springtime, warming and autumn–wintertime phases of incubation in which T, S and DO were adjusted (Figure 4A). All the eggs isolated from 12 June (start of winter) hatched during the springtime phase (after 60 days) when they were exposed to low temperatures (5.5 °C) (Figure 4B). A low % HBEEa (20%) was observed in eggs isolated from 5 September (the end of winter) during the springtime phase and a high percentage of these eggs were confirmed as non-viable by the neutral red test. This low number of viable eggs hatched after 180 days of incubation under favourable conditions for the species (springtime phase). In real time and under the same conditions of incubation, these eggs could hatch during March–April (autumn season). *Eurytemora americana* eggs isolated from 20 September, 2 October and 8 October (spring season) did not hatch during the springtime and warming phases (Figure 4B). The emergence of the first *E. americana* nauplii in these samples occurred in autumn–wintertime after the complete warming phase,

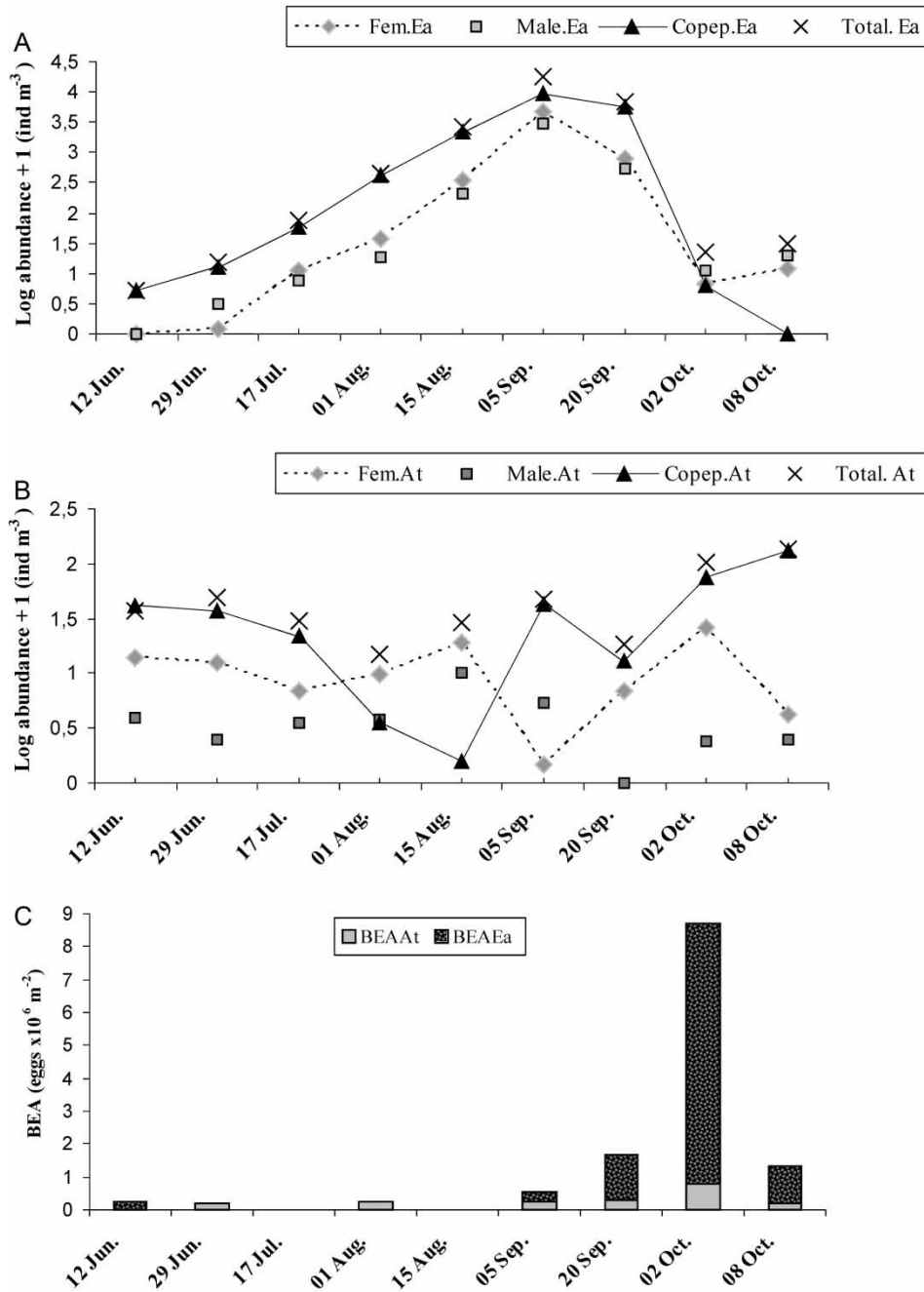


Figure 3. Temporal variation of population abundance and benthic eggs in the two copepod species. (A,B) *Eurytemora americana* and *Acartia tonsa* population dynamics and structure. (C) Abundance of benthic calanoid eggs (BEA), expressed in m^{-2} . Average abundance of *E. americana* benthic eggs (BEAEa) and average abundance of *A. tonsa* benthic eggs (BEAAt).

when the experimental temperatures began to drop. These first hatchings were observed after 280–294 days of incubation at 17°C and 30–33 of salinity. These eggs could hatch in May–July (late autumn–winter), translating the hatching time to real time. The neutral red test indicated that 15–20% of these eggs corresponded to non-viable benthic eggs. Hatching success of *A. tonsa* was mostly recorded during the springtime phase, in eggs isolated from samples collected on 29 June, 1

August and 5 September (winter season) (Figure 4B). The emergence of the first *A. tonsa* nauplii occurred at 9 and 12°C after 60–70 days of the springtime phase. These eggs could hatch in September–November (spring season), translating the hatching time to real time. A delay in the hatching of *A. tonsa* eggs isolated from 5 September samples was also observed, reaching maximum hatching after 180 days of incubation at temperatures $\geq 15^\circ\text{C}$.

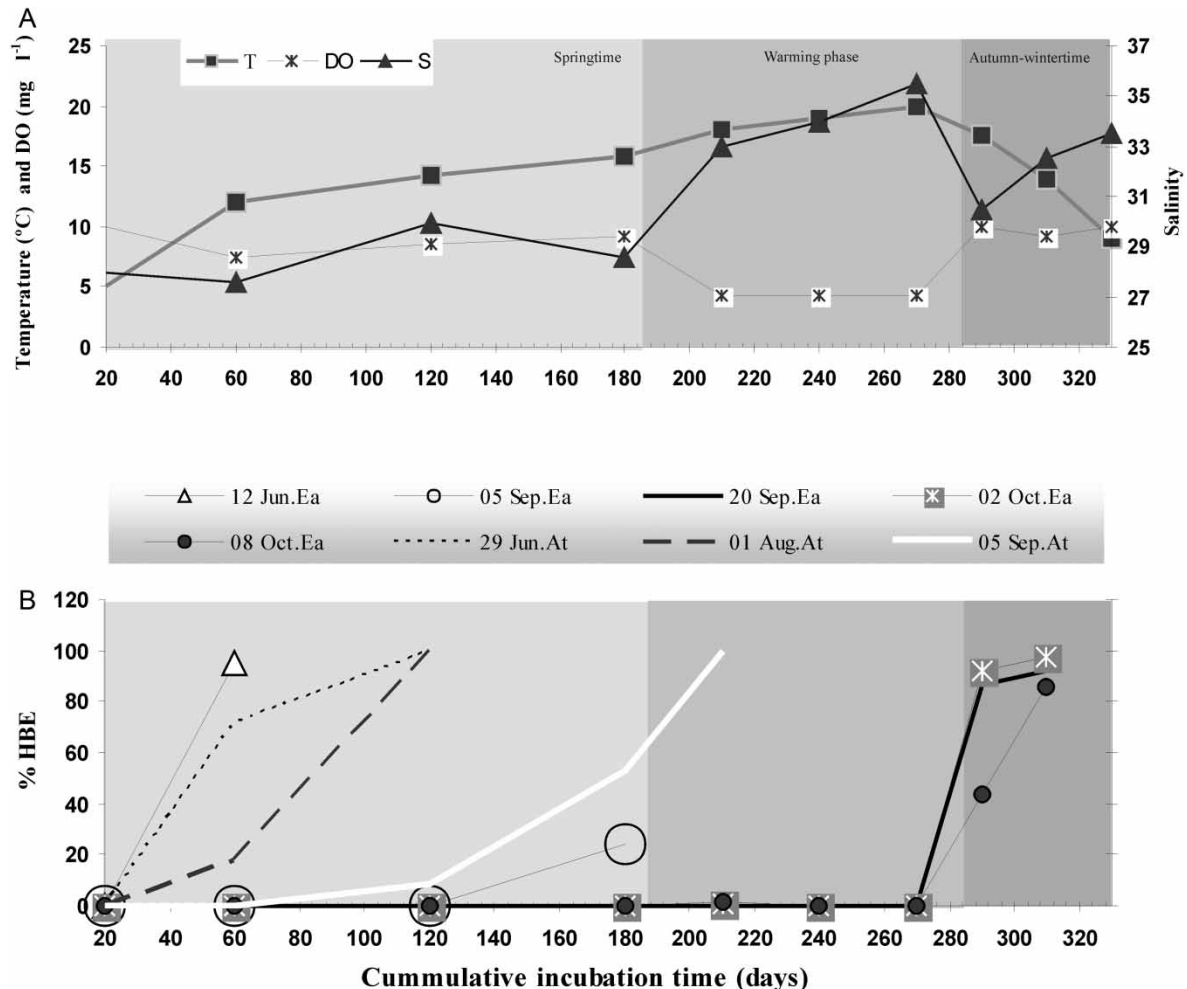


Figure 4. Incubations of benthic eggs. Incubation phases: springtime, warming period and autumn–wintertime, illustrated in three shades of grey. (A) Experimental conditions of the three experimental phases and (B) percentage of cumulative hatched benthic eggs (%HBE).

Discussion

The average abundance of calanoid copepod eggs found in the present study (1.85×10^6 eggs m^{-2} ; range 0.21 – 8.68×10^6 eggs m^{-2}) was higher than the values reported in Northern California coastal waters (Marcus 1995), the Tyrrhenian Sea, Ionian Sea and Adriatic Sea (Belmonte et al. 1995), the Bay of Biscay (Masero & Villate 2004), the Baltic Sea (Viitasalo & Katajisto 1994) and the Seine estuary (Glippa et al. 2011), but similar to those reported for the Inland Sea of Japan (Kasahara et al. 1975) and Malaga harbour of Spain (Guerrero & Rodríguez 1998). The higher abundance of calanoid eggs observed in the present study compared to previous data from the same area (Diodato et al. 2006) could be due to variations in the isolation techniques used. In the present work we used direct observation under a stereo microscope, whereas Diodato et al. (2006) followed the sucrose flotation technique (Onbé 1978) to isolate the eggs. Following this technique,

the sample is placed in a sugar solution (1000 g sucrose in 1000 ml distilled water) and centrifuged at 3000 rpm for 4 min, placing the eggs in the supernatant. We chose not to use the Onbé technique because it was observed that many eggs (incomplete egg clutches) were retained in the discarded sedimented phase.

In agreement with reports from other estuaries, the sinking of calanoid eggs probably follows the same dynamic settlement of silt–clay particles in zones of low tidal energy such as the innermost zone of the BBE (Marcus & Fuller 1989; Lindley 1990; Cuadrado et al. 2004). In addition to this, the greatest abundances of both species occur in the inner zone of the estuary and population recruitments are observed annually, firstly in this zone and then in the external ones (Hoffmeyer 1994, 2004; Hoffmeyer et al. 2008). The retention of *Eurytemora americana* and *Acartia tonsa* in the head of this estuary could therefore be explained not only by

the current dynamics and water circulation patterns (Hoffmeyer et al. 2009; Menéndez et al. 2011), but also by the sedimentation process of resting eggs as it occurs with suspended sediments in the innermost zone (Cuadrado et al. 2004).

Eurytemora americana showed a higher abundance of benthic eggs than *A. tonsa* throughout the entire study period, particularly during the last field sampling days; the benthic egg abundance of *A. tonsa* was consistently low. The differences in the abundance of benthic eggs of both species would be given by the type of dormancy eggs that both species display. *Eurytemora americana* eggs would pass through a refractory period taking a long time to hatch; therefore dense banks can generate, probably enhanced by recurrent sedimentation and resuspension events, like clay particle deposition on muddy areas. *Acartia tonsa* eggs, however, may not exhibit a refractory phase, taking less time to hatch and therefore not accumulating over time in high sedimentation zones.

The seasonal pattern of benthic egg abundance found in this study would be subject to the low spatial variability of the sampling and the re-suspension–deposition processes in the sediments is patchy, which unfortunately have not been measured. Nevertheless, our results are likely to reflect an apparent relationship between benthic egg abundance and *E. americana* population dynamics. At the beginning of the population pulse (12 June) a low abundance of *E. americana* eggs was found, with no adults in the water column. The high hatching success (90–100%) observed in benthic eggs from this sampling date after they were subjected to acclimation and springtime conditions suggests that they probably came from previous planktonic pulses, passing through a natural refractory period during previous summers. Moreover, it is supposed that recruitment from benthic eggs began before the sampling took place because copepodites were observed on 12 June. The absence or low amount of non-viable benthic eggs of *E. americana* in samples collected during the period of population growth (from 29 June to 15 August) could be explained by this species' reproductive strategy, involving only subitaneous egg production during this period (Berasategui et al. 2012). The non-viable benthic eggs found during this period may therefore be subitaneous eggs. According to studies undertaken during the same year (Berasategui et al. 2009a, 2012), *E. americana* females produce larger clutch sizes of subitaneous eggs, which have high hatching success during the period of population growth. This reproductive strategy coupled with the recruitment of eggs from previous pulses triggers the exponential population increase. The low abundance of *E. americana*

benthic eggs found in the samples collected on 5 September (population peak) could be explained by a reproductive–metabolic shift as reported for this species (Berasategui et al. 2009b). During this transitional period the females begin to produce diapause eggs along with subitaneous eggs (Berasategui et al. 2012), supplying the egg bank with diapause eggs from 5 September, thus ensuring the species recruitment in future years once they have disappeared from the water column in October. A decrease in the abundance of benthic eggs was registered after 2 October, which could be explained in part by the low abundance of females in the water column ($<10 \text{ ind. m}^{-3}$) and low production of diapause eggs per sac (Berasategui et al. 2012).

The results of the incubations carried out with *E. americana* benthic eggs collected after the population peak indicate that the first nauplii appeared at temperatures around 17°C after passing through the warming phase (280 days of incubation). The eggs were observed to hatch rapidly at lower temperatures (17–15°C) and higher salinities (30–32). If they had been quiescent, they would have hatched during the first phase of the acclimation period (Marcus 1996), under similar conditions of temperature and salinity. In order to be able to hatch, these eggs need to pass through a refractory period prior to the return of favourable conditions, in agreement with the findings on diapause in eggs of the congener *Eurytemora affinis* and other calanoid species (Grice & Marcus 1981; Ban 1992; Viitasalo 1992; Castro-Longoria & Williams 1999; Katajisto 2006). According to Hoffmeyer et al. (2009), the first *E. americana* nauplii recruited from benthic eggs in the wild were detected in the water column of the BBE during the cold season, at surface temperatures $\leq 6-7.2^\circ\text{C}$ and salinities of 27–30. In agreement with these authors, in our studies a high hatching rate of benthic eggs from samples collected on June 12 was recorded at 5°C. These eggs presumably come from previous plankton pulses and have passed through a refractory phase in the wild. However, our findings also showed that after the experimental refractory phase, hatching can start at higher temperatures (17°C) than expected ($\leq 6-7.2^\circ\text{C}$). This finding is surprising, as the disappearance of the population from the water column was recorded at $> 15^\circ\text{C}$ (Hoffmeyer 2004; Hoffmeyer et al. 2009). This probably indicates that the maximum limit of temperature tolerance by *E. americana* is higher than that registered in the wild. Although low temperatures ($\leq 17^\circ\text{C}$) may promote the hatching of *E. americana* benthic eggs after a refractory period, the main determinant of diapause duration seems to be the maternal effect. As mentioned by Belmonte & Pati (2007), females may pre-establish the duration

of the diapause in their eggs, and the eggs do not perceive external stimuli during the refractory phase. The field recruitment of *E. americana* could be affected by additional variables such as sediment resuspension frequency (Siokou-Frangou et al. 2005), anoxic conditions (Grice & Marcus 1981; Ban & Minoda 1992; Marcus et al. 1997) and predation on eggs and nauplii by benthic organisms (Albertsson & Leonardsson 2000). In fact, predation on *E. americana* nauplii by fish larvae of *Rammogaster arcuata* may be taking place, because this copepod has been mentioned as the main food item in studies on stomach content of this fish in the BBE (Lopez-Cazorla et al. 2011). All these factors could therefore contribute to the late detection of *E. americana* nauplii in the water column (Hoffmeyer et al. 2009) under the lower cold season temperatures (June to August) than those recorded in our study, which resemble early autumn temperatures. However, Menéndez et al. (2012) observed a limited presence of *E. americana* nauplii in bottom water samples from Cuatrerros Port from April to May, before the cold season, which supports our hypothesis that the hatching of benthic eggs commences before the onset of the cold season.

In the case of *A. tonsa*, a low abundance of benthic eggs was observed in the samples collected during the cold season (from 12 June to 5 September) and higher abundances were detected in the sediments of spring samples, probably as a result of accumulation of eggs during previous autumn and winter. The low abundance and the absence of benthic eggs observed in samples collected during the cold season (from 12 June to 5 September) could be explained in part by the low abundance of adults in the water column and by the very low temperatures recorded, unfavourable to the species' fecundity (Holste & Peck 2006). It could also be argued that the low temperatures prevailing during the cold period enable *A. tonsa* eggs to settle in the sediment, since the time required for egg development is longer than the sinking time (Castro-Longoria 2003). The results of the incubations carried out with *A. tonsa* show a trend in the hatching pattern that should be reaffirmed in subsequent studies, considering the low number of incubated eggs and replicates. Taking this into account, it could be said that eggs produced by females acclimated to low winter temperatures showed delayed hatching at temperatures above 10°C. This hatching pattern of *A. tonsa* benthic eggs isolated from cold season samples coincides with the findings of Holste & Peck (2006) and Drillet et al. (2011). The same trend was also observed by Katajisto (2006), who reported delayed hatching at temperatures of 13–18°C in eggs with a

temporary cooling inhibition (to 3°C). Furthermore, taking into account that eggs found in samples after 5 September probably come from accumulated cold period lays which have been naturally trapped in the sediment for longer, these eggs would need a longer incubation time (120–180 days) and higher temperatures ($\geq 15^\circ\text{C}$) to exit from dormancy than those found on earlier dates. According to Castro-Longoria (2001) and considering the long time they required to hatch, these eggs could be classified as diapause eggs. However, we do not consider the *A. tonsa* eggs in the BBE to be diapause eggs in view of the high hatching success observed at 18–20°C immediately after spawning in eggs produced during the cold season (Berasategui, unpublished results). Indeed, the delayed hatching of *A. tonsa* resting eggs found in winter sediment samples of the present study is probably due to prolonged exposure to low oxygen and light conditions during natural storage in the sediments (Marcus 1980, 1996). In agreement with Katajisto (2006), our findings therefore suggest that *A. tonsa* displays an intermediate type of dormancy between diapause and quiescence, showing once again the plasticity of this species in adapting to its habitat. *Acartia tonsa* eggs found in the spring samples hatched mostly during the period of acclimation (within 30 days of incubation) and did not show a marked dormancy and like the eggs from the cold season, their hatching success after brief exposure to temperatures of 12–17°C and salinities of 27–30 was high (80–100%). Furthermore, fast hatching (24–48 h) was observed when the eggs were exposed to the same salinity and temperatures of 17–20°C. According to the findings of Chen & Marcus (1997) and Katajisto (2006), these eggs could therefore be subitaneous, quiescent or, in the case of unhatched eggs, non-viable, suggesting that both subitaneous and resting eggs are present in the sediments of the BBE.

Resting benthic egg banks in the inner zone of the BBE seem to provide temporary refuge for both calanoid copepod species under adverse environmental conditions (i.e. warm season for *E. americana* and cold season for *A. tonsa*), thereby contributing to their survival and playing an important role as dispersal agents in coastal environments.

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