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ORIGINAL ARTICLE

Reproductive trade-off of the copepod *Acartia tonsa* in a hypersaline estuary of the Southwestern Atlantic. Temporal variations in the morphology of eggs

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ABSTRACT

The purpose of the present work was to study the seasonal variations in egg production, morphology and hatching success in the cryptic species *Acartia tonsa*, taking into account variations in female size, population abundance and environmental factors in a turbid and hypersaline estuary. Sampling was performed during the austral warm (18–23°C and 32–36 salinity) and cold seasons (5–7°C; 32–38) in Bahía Blanca Estuary (BBE), Argentina, during 2007 and 2009. Field-collected females were incubated in the laboratory simulating *in situ* environmental conditions, and specimens from fixed samples were measured using optical and scanning electronic microscopy. *Acartia tonsa*'s marked seasonality in its reproductive traits was found to ensure its permanence in the water column all over the year. During the warm season, small-sized females were observed to invest their energy in the production of subitaneous eggs with high hatching success and smooth appearance (12.95 ± 2.38 eggs f^{-1} day^{-1} and specific egg production rate (SEP) of 16.57%C f^{-1} day^{-1}). During the cold season, females invested C in body mass as well as in the production of resting eggs of three different morphotypes (6.56 ± 3.2 eggs f^{-1} day^{-1} and SEP of 7.37%C f^{-1} day^{-1}). Although these morphotypes were found to show differences in surface ornamentation, they exhibited the same delayed hatching behaviour. The eggs with shorter spines were found to integrate the resting egg bank in BBE. Our findings confirming a delayed egg hatching behaviour and a great tolerance to low temperatures and high salinities in the *A. tonsa* population in BBE suggest that this possible strain is a valuable phenotype for aquaculture.

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Introduction

Acartia tonsa Dana, 1849 is the best-known species among small marine copepods. It has a cosmopolitan distribution along warm coastal areas and it is an important link in natural trophic webs. This copepod is resistant to varied culture conditions and it has a beneficial nutritional profile for aquaculture purposes (Drillet et al. 2006). However, *A. tonsa* is a cryptic species that includes genetically divergent lineages, which may have specific habitat or culturing requirements (Hansen et al. 2010a; Drillet et al. 2011a). Previous research on different *A. tonsa* populations has shown that both genetic differentiation and specific strain differences in the life-history traits of this species exert their influence on egg size, egg production, hatching success, biochemical contents of eggs and adults and egg survivorship following cold storage (Drillet et al. 2008a, 2008b).

Further studies on wild and cultured *A. tonsa* populations from the northern hemisphere for which the effect of temperature on body and egg size have been analysed demonstrated an inverse relationship between these variables (Ambler 1985; Hansen et al. 2010a). A synergistic effect between low temperatures and low quantity–quality of food was observed not only to decrease egg production but also to induce resting egg production in this cryptic species (Uye & Fleming 1976; Dutz et al. 2004; Drillet et al. 2011b). The presence of subitaneous eggs and of two types of resting eggs, whose classification was based on their physiological response, was reported in different populations of *A. tonsa*.

Subitaneous eggs are normally produced to ensure population recruitment in the short term and they have the ability to hatch rapidly (i.e. in general, subitaneous eggs hatch within 72 h; Grice & Marcus 1981). These eggs may become quiescent (i.e. they have a break in embryogenesis) when they are exposed to adverse environmental conditions, such as low temperatures, anoxia or low dissolved oxygen concentrations, low salinities (0–5 ‰) and decreased photoperiod (≤12 h light) (Marcus et al. 2004; Peck &

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Holste 2006; Højgaard et al. 2008). The development of subitaneous eggs may resume as soon as favourable conditions are restored. In contrast, diapause eggs are in a state of arrested embryonic development and do not hatch, even under favourable conditions, until they complete a refractory phase (Grice & Marcus 1981; Marcus 1987). Eggs known as 'delayed hatching eggs' hatch over an extended period compared to quiescent eggs (< 2 months) and do not need to undergo a refractory period to hatch (Chen & Marcus 1997). The production of these eggs was reported in *A. tonsa* populations from the Baltic Sea during winter conditions, cold storage and food limitation (Katajisto 2006; Drillet et al. 2011b).

In spite of several heterogeneous descriptions of the morphology of eggs, many researchers have agreed on the description of *A. tonsa*'s spiny eggs according to which spines are longer in resting eggs than in subitaneous eggs (Marcus 1990, 1991; Belmonte 1992; Katajisto 2006; Hansen et al. 2010b). Based on this observation, morphology could be used to identify egg types, differentiating resting eggs from subitaneous ones (Belmonte et al. 1997).

In the Bahía Blanca Estuary (BBE), *A. tonsa* is a relevant species within the holoplanktonic fraction given its central role in the trophic web, its high abundance and its wide seasonal distribution (Hoffmeyer 2004). This copepod is found in the water column throughout the year, with maximal abundance during the warm season (austral summer: December to March) and minimal abundance during the cold season (austral winter: June to August), when temperature decreases and the environment becomes unfavourable for the species (Sabatini 1989; Hoffmeyer 1994, 2004). Although females in BBE have shown seasonal variation in their body size (Sabatini 1989; Hoffmeyer & Torres 2001), the relationship between body size and reproductive traits has not yet been addressed. Likewise, although the production of subitaneous eggs with different spine lengths in this estuary has also been reported (Diodato et al. 2006), its relationship with the size of females, the production of morphotypes relative to embryogenesis and resting stages has not yet been elucidated.

Previous research reported a low production of subitaneous eggs as well as the production of resting eggs in BBE during the cold season (Sabatini 1989; Hoffmeyer 2004). *Acartia tonsa* spiny eggs were found in the bottom sediments of BBE (Diodato et al. 2006). They showed delayed hatching after experimental incubation under favourable conditions (14–20°C and 27–33 of salinity) (Berasategui et al. 2013). Therefore, although these findings lead us to hypothesize that spine length is longer in resting eggs, this hypothesis has not been confirmed to date.

On the other hand, in spite of the relevance of *Acartia* spp. in aquaculture and in the trophic dynamics of coasts and estuaries, no studies on the fecundity of the *A. tonsa* population in BBE have so far been conducted. An integral study on the seasonal reproductive pattern in the pelagic and benthic phases is therefore necessary to understand the reproductive traits of this copepod. In line with this, the purpose of the present work was to examine the seasonal variations in the production, hatching and morphology of eggs in wild *A. tonsa* females taking into account the variations in the principal environmental factors, such as female size and population abundance in a Southwestern Atlantic estuary. This study will contribute to current knowledge on adaptive fecundity in a 'possible *A. tonsa* strain' living in a hypersaline environment, and will provide innovative requirements for the development of monocultures of this cryptic species.

Materials and methods

Study area

The Bahía Blanca estuary (BBE) (38°45′S; 62°22′W) is a mesotidal coastal plain ecosystem located on the Atlantic coast of Argentina (Figure 1). Sampling and *in situ* measurements were performed in Puerto Cuatreros, a fixed station located in the innermost area of BBE. The inner zone of the estuary shows a marked seasonal variation in water temperature (5–27°C) over the year (Freije et al. 2008). Average salinity is 33 even though values as low as 17.3 and as high as 41.9 have been recorded in the inner zone of the estuary. Hypersaline conditions are usually registered during warm seasons with low precipitation levels (Freije et al. 2008). Suspended sediment concentrations vary between 30 and 400 mg l^{-1} and result mainly from the erosion of tidal flats and island shores (Perillo et al. 2001). The annual mean concentration of dissolved oxygen is close to 7 mg l^{-1}, reaching 13 mg l^{-1} during periods of phytoplankton blooms (winter and late summer) (Freije et al. 2008; Popovich & Marcovecchio 2008).

Phytoplankton dynamics in BBE have been thoroughly studied over the past three decades and the annual cycle has been characterized by a diatom bloom belonging to the genera *Thalassiosira* and *Chaetoceros*, being followed in importance by nano-phytoflagellates (maximum abundance in summer) and dinoflagellates (maximum abundance in autumn and spring). The annual cycle of phytoplankton biomass is

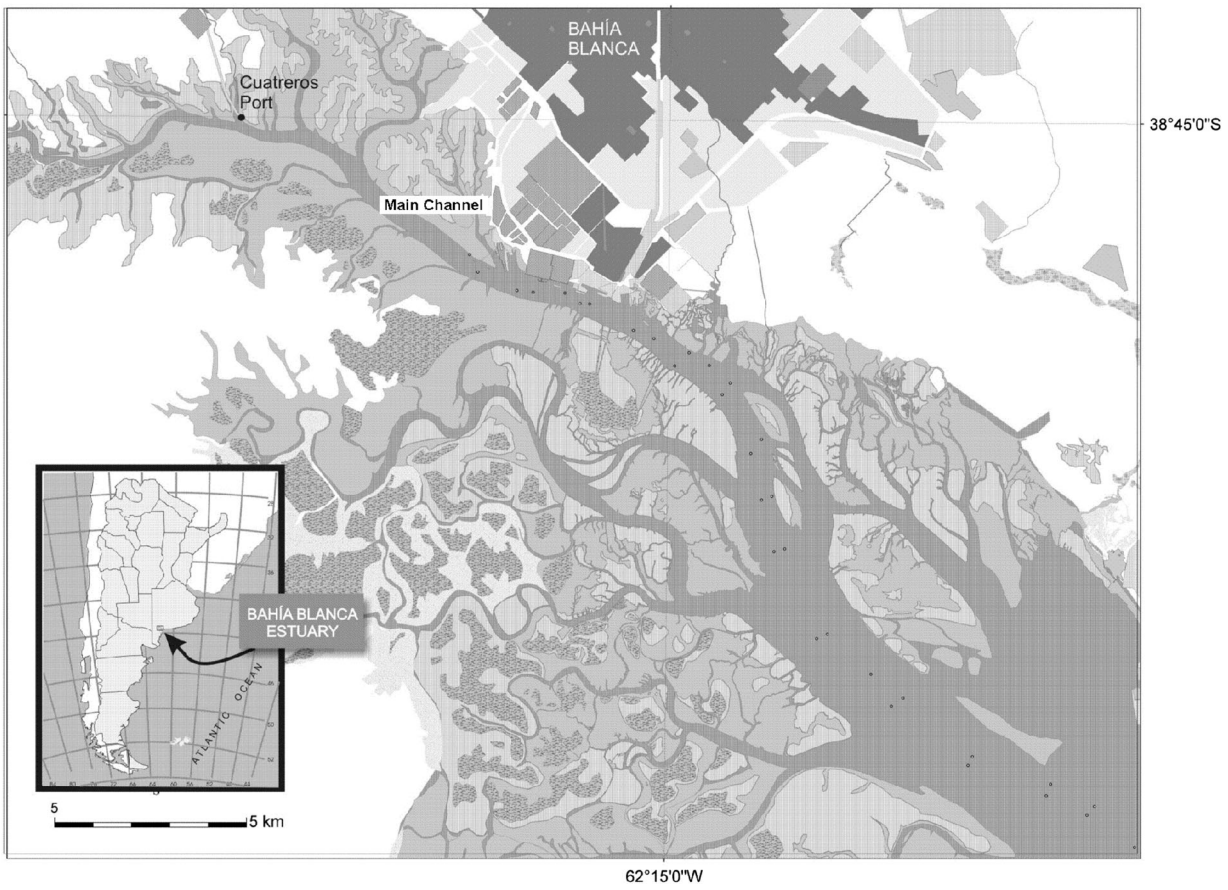

Figure 1. Map of Bahía Blanca Estuary (BBE) showing the locations of Bahía Blanca City and of the sampling site, Puerto Cuatreros.

characterized by a recurring diatom bloom in winter–early spring, which represents the most important annual event of productivity in terms of chlorophyll concentration, cell density and biomass. A chlorophyll concentration of up to 25 mg l^{-1} has been recorded, mainly during the blooming event (Guinder et al. 2010), *Thalassiosira curviseriata* Takano being the typically dominant winter species blooming for over 20 years (period 1978–2002). Small phytoflagellates and some dinoflagellates (< 20 µm) were observed during the blooming period, but neither of their abundances ever reached 10% of total phytoplankton abundance (Guinder et al. 2010).

Field procedures

Sampling was carried out in BBE on seven days during 2007 and 2009, covering the warm season (13 March 2007, 12 March 2009, 1 April 2009) and the cold season (29 June 2007, 17 July 2007, 1 August 2007, 15 August 2007). On each sampling day, two mesozooplankton samples were collected by oblique tows in the surface layer (0.5–2 m depth water) using a plankton net with 200 µm mesh provided with a HydroBios mechanical flow metre. One sample was preserved in 4% formalin to estimate the population abundance (PA) of the species. The other sample was kept alive and was transported in a thermally insulated container to maintain the field temperature filled with 5 l of *in situ* seawater. Additionally, 20 l of seawater were collected for the incubation medium (filtered through a sieve of 60 µm pore size) and for chlorophyll-*a* (Chl *a*) determination (Lorenzen 1967) (using a Van Dorm bottle). Surface temperature (TEMP), water density (DEN) and salinity (SAL, measured in practical salinity units) were recorded using a HORIBA U-10 multisensor at the same time of sample collection.

Laboratory procedures

The fixed mesozooplankton samples were washed with seawater filtered (0.75 µm) through a sieve of 135 µm to remove formaldehyde. The quantitative–qualitative analyses of these samples were carried out in a Bogorov chamber under a Wild M5 stereoscopic microscope (SM). Following the methodology proposed by Boltovskoy (1981), counting was done either in the complete sample or by aliquots (10% of the sample

using a Hensen–Stempel plunger sampling pipette of 5 ml) depending on the abundance of the sample. Total PA of *Acartia tonsa* was estimated as the total number of individuals per cubic metre (ind. m^{-3}). Abundance of males, females and copepodites was also recorded.

Live mesozooplankton samples were diluted using *in situ* seawater ≤ 60 µm and transferred into plastic containers of 10 litres. Adult specimens were subsequently selected for experimental incubation.

Egg morphology, egg production rate and female size

To analyse these variables, two experimental incubations were performed on each day. In each experimental device, 20 females (adults < 50 ind. l^{-1}, Peck & Holste 2006) were placed in Petri dishes with 500 ml of seston ≤ 60 µm. Incubations without acclimatization were performed simulating *in situ* temperature and photoperiod conditions (12–16 h light) in a culture room over 24 hours. After this period, the health status of females was verified and the whole sample was subsequently fixed in 4% formalin. The eggs in these fixed samples were counted to estimate the egg production rate (EP). Total length of females was measured (FS; $n = 40$ females by sampling date) under a stereo microscope. EP was expressed as the number of eggs per female alive per day or µgC f^{-1} day^{-1} using the conversion of Uye (1981). The reason why females were not acclimated was to show a simulation of their physiological responses in the natural environment (Tester & Turner, 1990).

Egg morphology was analysed and the relative proportion of each morphological type (based on spine length from the shortest to the longest, e.g. EI, EII, EIII) in relation to total egg production was estimated (525 eggs were analysed during the cold season and 779 eggs were analysed during the warm season). Egg diameter including the spines (ES, as total size of egg) and egg diameter excluding the spines, were measured by optical microscopy (OM) ($n = 15$–25 egg by morphological type, per sampling date). A subsample of 15–20 eggs for each morphological type in each study season was also selected for scanning electronic microscopy (SEM). These eggs were pre-fixed in vials with 4% formalin for one month (Belmonte & Pruce 1994). After pre-fixation, they were prepared for SEM following Sorrivas de Lozano & Morales (1986) and Castro-Longoria (2001) SEM protocols. The eggs were subsequently rinsed in seawater (≤ 0.2 µm) and then cleaned by ultrasound in 1% hydrochloric acid solution for 6 min. Post-fixation was overnight at 18–20°C with formalin acetic acid ethanol. The eggs were subsequently dehydrated in an ethanol series and dried until critical point. They were finally mounted on stubs and sputter-coated with gold.

Specific egg production rate (SEP) per day was calculated following Drillet et al. (2008b). It represents the quantity of carbon used per day for egg production, which is divided by the carbon weight of females. Egg carbon content was calculated indirectly, i.e. using the spineless egg diameters measured and the equation $W = 0.14 \times 10^{-6}$ µg C μm^{-3}, where W is the egg volume content of carbon (µg C) (Uye 1981; Kiørboe et al. 1985). The carbon content of females was calculated using the female's cephalothorax length measured using the equation $W = 1.11 \times 10^{-5} \times L^{2.92}$, where W is the female body weight (ng C) and L is the prosome length in micrometres of females (Berggreen et al. 1988).

Because the data collected did not provide evidence of a normal distribution, the Mann–Whitney test was used to analyse the average differences that EP, FS, and ES variables showed between the warm and cold season. To complement the information on *Acartia tonsa* reproductive traits, the relationships between the environmental variables EP, FS, ES and PA were analysed using Spearman's rank correlations.

Hatching success vs. egg morphology

An experimental incubation simulating *in situ* temperature and photoperiod conditions was performed on a daily basis to analyse egg hatching. Each experimental device was developed in the same way as stated above. After 24 h incubation, adults were removed and eggs were counted and classified according to their morphology into EI, EII and EIII under OM (at the beginning of incubation $t = 0$). Eggs were then placed in incubation devices for 7 days. After the incubation time ($t = 7$), the hatching success of each morphological egg type was evaluated by counting the number of nauplii present in each device expressed as a percentage, taking into account the initial number of each egg morphotype produced (%H.EI, %H.EII, %H.EIII).

Results

Environmental conditions and population abundance

Acartia tonsa abundance (14.07–133.59 ind. m^{-3}) was observed to be lowest during the cold season, with temperatures ranging between 5 and 7°C and salinities

ranging between 32 and 38. The scarcity of adults from mid July to mid August (♀ 8.64 ± 2.7 ind. m^{-3}, ♂ 2.29 ± 0.68 ind. m^{-3}, copepodites 19.86 ± 16.69 ind. m^{-3}) coincided with the lowest temperatures (~5.25°C) and high salinities (~36.62) recorded *in situ* (Figure 2 (A)). In contrast, during the warm season (18–23°C and 32–36), population abundance ranged between 378.26 ind. m^{-3} and 1480.54 ind. m^{-3} (Figure 2(B)).

Egg morphology, egg production rate and female size

Females from the cold season were observed to have a significantly larger sizes than females from the warm season (Mann–Whitney $U = 4369$, $P \ll 0.001$) (Figure 2C). Females produced, in general, three different morphological egg types throughout the study. They were described taking into account their spine length from OM and SEM as EI: ≤ 3 µm, EII: 4–12 µm and EIII: 13–22 µm (Figure 3). Significant differences in ES were detected in both seasons ($U = 1605$, $P \ll 0.001$). In the cold season, eggs were found to have larger spines and diameters ($n = 180$, ES 90.21 ± 0.92 µm; diameter without spines 87.97 ± 6.74 µm) than those produced during the warm season ($n = 45$, ES 84.37 ± 0.14 µm, same diameter and smooth appearance by OM) (Figure 2D). The estimated volume of EII eggs ranged from 0.00057 to 0.00064 mm^3 including the spines, whereas in spineless eggs it ranged from 0.00032 to 0.00036. The average virtual volume of spines of EII eggs represented 44% of the total volume of the egg ($n = 60$). The estimated volume of EIII eggs ranged from 0.00092 to 0.00105 mm^3 including the spines, whereas in spineless eggs it ranged from

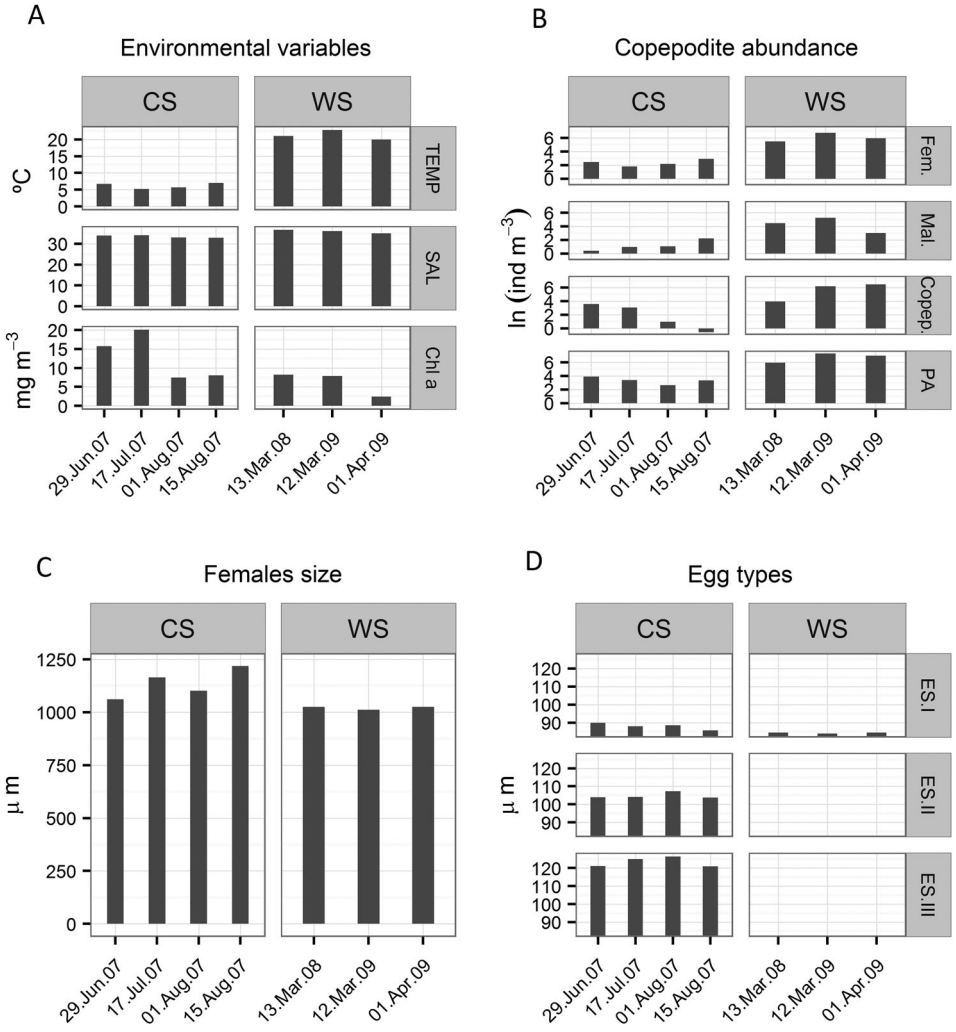

Figure 2. Environmental conditions and biological variables studied in warm (WS) and cold (CS) season. (A) Environmental conditions registered *in situ* and simulated in experimental devices (TEMP, temperature; SAL, salinity measured in practical salinity units; and Chl *a*, chlorophyll-*a*). (B) *Acartia tonsa* abundances (Fem, abundance of females; Mal, abundance of males; Copep, abundance of copepodites; PA, total population abundance). (C) Seasonal size of females. (D) Total size of eggs including the spines (ESI, egg size of morphotype I; ESII, egg size of morphotype II, and ESIII, egg size of morphotype III).

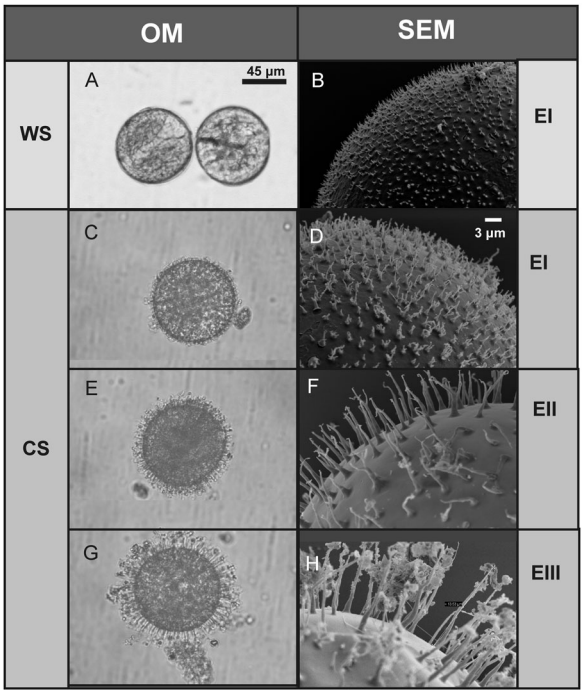

Figure 3. Seasonal morphology of *Acartia tonsa* eggs by optic microscopy (OM) and scanning electronic microscopy (SEM). Egg morphotype as EI: spine length ≤ 3 µm, EII: spine length 4–12 µm, and EIII: spine length 13–22 µm. (A) and (B) warm season (WS); (C–H) cold season (CS).

0.00034 to 0.00037. The average virtual volume of spines of EIII eggs represented 64% of the total volume of the egg ($n = 60$). No significant differences between mean EP values were observed in both seasons ($U = 18$, $P = 0.057$). In the cold season, a total egg production of 6.56 ± 3.2 eggs f^{-1} day^{-1} (0.32 µgC f^{-1} day^{-1}) was recorded. The eggs produced during this period were represented as 23–51% of EI and EII, respectively, whereas 15–26% of the total eggs were represented by EIII (Figure 4). SEM images showed branched spines in all these egg morphotypes (Figure 3 from C to H). During the warm season, a total egg production of 12.95 ± 2.38 eggs f^{-1} day^{-1} (0.57 µg C f^{-1} day^{-1}) was registered and only EI eggs were produced. SEM images of this egg morphotype showed small spines (1.7 µm) with a simple conical structure (Figure 3A,B). SEP ranged from 1.87 to 19.94% C f^{-1} day^{-1} throughout our study, an average value of 7.37% C f^{-1} day^{-1} and 16.57% C f^{-1} day^{-1} being estimated for the cold and warm season, respectively (Figure 4). ES showed a significant positive correlation with FS and DEN as well as a negative correlation with TEMP and PA. FS showed a significant negative correlation with PA, TEMP, SAL and EP. Furthermore, EP showed a significant positive correlation with TEMP and SAL (Table I).

Hatching success vs. egg morphology

During the cold season, low hatching success (9–40%) was observed in the three above-mentioned morphological types after 7 days of incubation. In contrast, during the warm season hatching success was very close to 100%, the first naplius larvae being recorded 24 h after egg laying (Figure 5).

Discussion

Large females, very low egg production, low egg hatching success and low abundance of *Acartia tonsa* in the water column were observed in our study, particularly at temperatures ranging between 5.2 and 7.6°C and salinities ranging between 32.7 and 37.5. This trend could be translated as a metabolic balance in favour of body mass with resting egg production during the

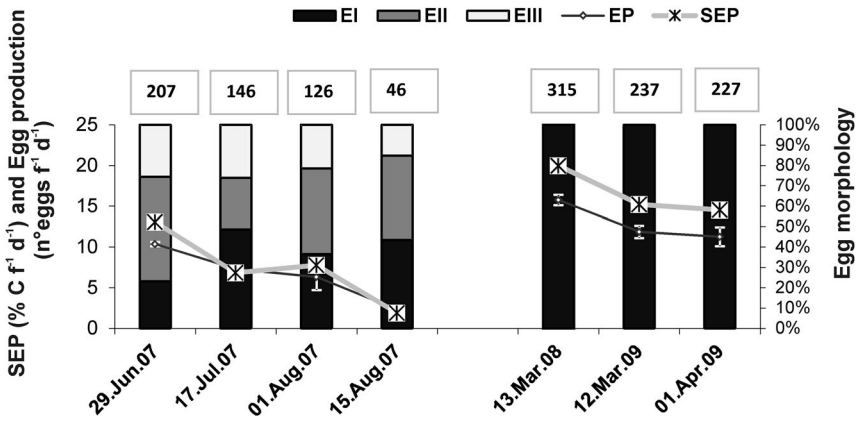

Figure 4. Seasonal egg production and percentage of each egg morphology. The percentage of each morphotype in relation to the total eggs produced was expressed as EI (% egg belonging to morphotype EI), EII (% egg belonging to morphotype EII), EIII (% egg belonging to morphotype EIII). The numbers above each column represent the total number of eggs produced and analysed (100%). EP, egg production rate per female per day; SEP, specific egg production.

Table I. Results of Spearman rank correlation. Abbreviations: see text.

Spearman rank order correlations	ES	FS	TEMP	SAL	CL-A	DEN	EP
FS	*0.74*						
TEMP	***−0.95***	*−0.79*					
SAL	−0.68	*−0.86*	0.64				
CL-A	0.29	0.29	−0.39	0.00			
DEN	***0.98***	0.77	−0.99	−0.68	0.31		
EP	−0.72	*−0.93*	*0.72*	*0.96*	−0.04	−0.74	
PA	*−0.88*	*−0.86*	0.79	*0.82*	−0.18	*−0.83*	*0.86*

Italic values are significant results $P \leq 0.05$ and bold italic values are significant results $P \leq 0.001$.

cold season in agreement with the SEP rate values obtained. The positive correlation between female size and egg size and the inverse correlation of both with temperature are consistent with those reported in previous studies on cultured copepods (e.g. Heinle 1969; Uye & Fleminger 1976; Ambler 1985; Kiorboe & Sabatini 1995). The seasonal variations in female size could be attributed to the seasonal variations in temperature and in the typical food conditions in BBE (Hoffmeyer & Torres 2001). Lower temperatures lead to a less-costly metabolism as well as a lower reproductive activity (Gaudy et al. 2000; Holste & Peck 2006; Hansen et al. 2010a). A metabolic balance in favour of body mass development could also be related to a phenotypic plasticity of the BBE population in response to low temperatures on account of the fact that smaller specimens are most vulnerable and have lower survival rates (Hansen et al. 2010a). The presence of large females may also be related to a longer intermoult duration, slow larval development and late maturity of copepodites V (Kiørboe & Hirst 2008; Hansen et al. 2010b). This agrees with a greater generational duration (~7 weeks) reported by Sabatini (1989) in *A. tonsa* populations in BBE during the cold season.

Although no significant differences in egg production were observed between the cold and warm seasons, a positive relationship between egg production and temperature was found in our study. The environmental conditions observed during the cold season could be considered relatively extreme, as it is known that in several populations of *A. tonsa*, egg-laying decreases under temperatures below 17 ± 2°C and salinities above 30 (Ambler 1985; White & Roman 1992; Castro-Longoria 2003). Holste & Peck (2006), working in experimental incubations with females from the northern Baltic Sea, detected no egg production in specimens incubated under the following conditions: 5.2°C, salinity of 18 and high food availability. Nonetheless, cold temperatures in our study seemed not to severely affect egg production. On the other hand, the possibility of a negative effect of high salinity on egg production (Calliari et al. 2006; Holste & Peck 2006) could not be assessed in our study as a result of the positive relationship found between EP and SAL. This finding could be related to the ability of adaptation of the BBE population to the natural high-salinity values of the estuary which are, in general, above 30 and may reach values of 40 during hot and dry summers (Freije et al. 2008). Further experimental studies under controlled salinity gradients are nonetheless necessary to support this hypothesis.

Although the nutritional condition is probably key to sustaining the reproductive activity in copepods (Kleppel & Hazzard 2000; Runge & Roff 2000; Kimmerer et al. 2005; Lee et al. 2006), no positive relationship between Chl *a* and egg production or between Chl *a*

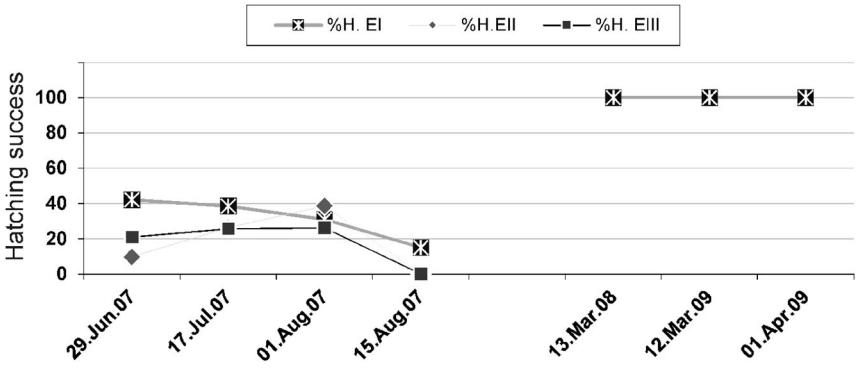

Figure 5. Hatching success expressed in % taking into account the different morphotypes of eggs produced (% H.EI, % H.EII and % H.EIII).

and females size was found, as expected, in our study. This is probably due to the fact that the reproductive performance of females is more related to the individual's past feeding history (Tester & Turner 1990) than to the current concentration of food in the water column. Likewise, this is probably related to the omnivorous preference of the species (Kleppel 1993), which exceeds the autotrophic fraction indirectly determined by Chl *a* in this study. The period studied in our research was part of an unusual year that showed lower turbidity of the water column, higher salinities and lower temperatures with respect to other years (Guinder et al. 2009). Lower temperatures were partially explained by an Antarctic polar wave, which affected the south of South America, lowering the temperature of the estuarine water (Barros & Silvestri 2002; Silvestri 2005). As a possible consequence of this, changes in winter phytoplankton composition were found (Guinder et al. 2009) which could, in turn, trigger changes in trophic links including those associated to *A. tonsa*, affecting its food prey spectrum. A study carried out by Guinder et al. (2009) on the abundance and composition of BBE phytoplankton was performed during the same period as that of ours (January 2007–February 2008) and provided an overview of the availability and quality of natural food before its capture by *A. tonsa* females. Although in our experiments, the trophic spectrum was restricted to prey smaller than 60 µm (Berggreen et al. 1988), it is widely known that the reproductive response 24 h post capture of copepods is influenced more by the *in situ* conditions than by the experimental conditions applied (Tester & Turner 1990). Values above 8×10^6 cells l^{-1}, 24.5 mg l^{-1} of Chl *a* concentrations and biomass values above 393 mg C l^{-1} were recorded in winter 2007. The dominant species were *Chaetoceros* sp., *C. debilis* Cleve, *Thalassiosira* sp., *T. pacifica* Gran & Angst and *T. curviseriata*, the latter having lower abundance with respect to previous years (Guinder et al. 2010). These authors also reported conspicuous changes in the seasonal phytoplankton dynamics, such as the absence of the typical cold season bloom, which showed a trend to decrease in magnitude and changes in the timing. They also reported the replacement of the dominant blooming species, *T. curviseriata* (8–30 µm), and the appearance of novel blooming species, such as *Cyclotella* sp. (5–15 µm), during the cold season and *Thalassiosira minima* Gaarder (5–15 µm) during the warm season. Shifts in phytoplankton size-structure towards small-sized diatom species and the replacement of relatively large *Thalassiosira* spp. by small *Cyclotella* species and *Chaetoceros* species (Guinder et al. 2010) were also reported. These changes in the composition of food together with a trophic overlap with the invasive copepod *Eurytemora americana* Williams, 1906 (Berasategui et al. 2009; Guinder et al. 2015) could have had a negative impact on food availability for *A. tonsa* during this particularly cold season in BBE.

The physiological response of hatching success observed in our study is in agreement with findings for *A. tonsa* populations from the Baltic Sea (Katajisto 2006; Drillet et al. 2011b). Our results reveal that *A. tonsa* produced subitaneous and resting eggs but not diapause eggs. The subitaneous eggs with smooth appearance (EI) were produced during the warm season and hatched in 24–72 h (Grice & Marcus 1981; Kiørboe & Sabatini 1994). Spiny eggs were produced during the cold season. They showed three morphotypes with different spine lengths and delayed hatching because they revealed a $\leq$40% hatching after one week under incubation conditions of 5–7°C and salinities of 32–38. Even though the hatching of these eggs was low, this could be indicative of the fact that they were not diapause eggs as described by Castro-Longoria (2001) in *A. tonsa* populations from Southampton, England because these eggs do not need a prior refractory phase to hatch. We can therefore conclude that such eggs did not appear to be subitaneous quiescent eggs, because they showed a delay in their hatching success (Chen & Marcus 1997; Katajisto 2006; Drillet 2010). This process was, in fact, observed particularly in the eggs produced in late winter with a more pronounced dormancy state, showing lower percentages of hatching after 7 days of incubation. Unfortunately, no complementary intravital staining technique was used in the present study to determine embryo viability in the unhatched eggs (60–80%) from the cold season. Therefore, the presence of decayed eggs among these unhatched eggs could only be assumed.

In our study, whereas egg surface was observed to be smooth shortly after eggs were spawned, spines of different lengths were found to emerge after incubation. This was in agreement with observations of other copepod eggs by Blades-Eckelbarger & Marcus (1992) and Hansen et al. (2010b). As stated above, three egg morphotypes with different spine lengths were observed during the cold season, whereas eggs that were smooth or had tiny spines were detected during the warm season. The same was observed after the experimental incubations performed with wild females from BBE during the cold and warm season of 2003 and 2004 (Berasategui 2010, personal observations 2007, 2008 and 2009; Figure 6). The same morphological variety of eggs was found in the

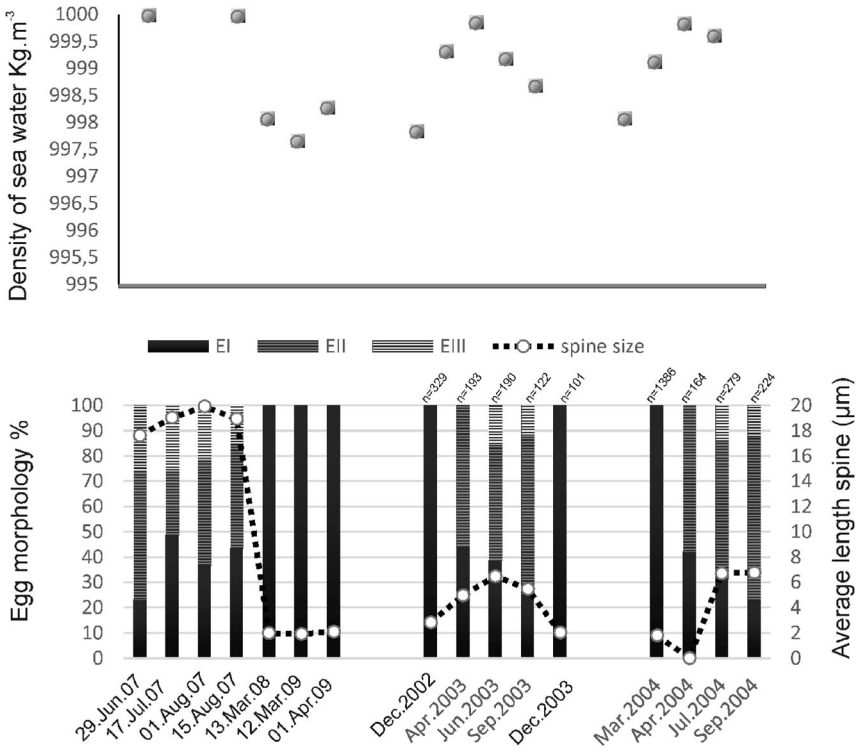

Figure 6. Seasonality in the morphology and spine length of *A. tonsa* eggs in relation to seawater density. Results from 2002, 2003 and 2004 are unpublished data from Berasategui (2010). The letter 'n' above each column indicates the total number of eggs observed on each day. Spine size represents the average spine length taking into account all morphotypes present on each day.

water and sediments from the bentho-pelagic interface in BBE (Diodato et al. 2006; Berasategui et al. 2013). This is indicative of the *A. tonsa* population's reproductive pattern in BBE. The comparison of findings regarding *A. tonsa* populations from the northern hemisphere with those from BBE reveals that only *A. tonsa* eggs from high latitudes (northern Europe) have characteristics similar to those studied in our work (see Table II). Although the variations in spine lengths is an interesting phenomenon, it is extremely complex

Table II. Morphology of *Acartia tonsa* eggs studied in the northern and southern hemispheres.

Authors	Study area	Source eggs	Surface appearance	Environmental condition	Egg diameter	Spine size	Type of egg
Marcus (1990)	California	Benthic eggs F incub.	Spiny	8.4–13.5°C 32.9–33.9 sal.	80.6 ± 1.8 and 81.0 ± 0.0 µm	18–25 µm	Diapause
Van Waveren & Marcus (1993)	Turkey Point, Mexico	F incub.	Spiny	16°C	60–112 µm	1.5–9.5 µm	Subitaneous
Chen & Marcus (1997)	Florida, USA	Benthic egg F incub.	Spiny Smooth	10–25°C 20–30 sal.	73.0–76.9 µm 77.8 ± 1.5 µm	<13 µm	Subitaneous quiescent
Belmonte (1998)	Venice lagoon, Italy	F incub.	Spiny	16–25°C 34 sal.	69.8–73.7 µm	3–7 µm	Subitaneous
Castro-Longoria (2001)	Southampton, England	F incub.	Smooth spiny	15–20°C 32 sal.	76.54–79.8 ± 2 µm	4.8 µm 6.8 ± 1.92 µm	Subitaneous Diapause
Hansen et al. (2010b)	Limfjord estuary, Denmark	F incub.	Smooth	2–12°C	80–86 µm	–	
Drillet et al. (2011b)			Short spines Truncated spines Long spines			5–15 µm <10 µm Up to 30 µm	Subitaneous quiescent Delayed hatching
Our study	Bahía Blanca, Argentina	F incub.	Smooth Short spines Long spines	5–20°C 30–36 sal.	85–93.5 µm	≤ 3 µm 4–12 µm 13–22 µm	Subitaneous Delayed hatching

to provide an explanation in support of it as a result of several factors that modulate egg ontogeny, such as environmental and maternal inheritance (Marcus 1991; Hansen et al. 2010b). In other calanoid species it has been observed that spine length in eggs is inversely correlated with water density but positively correlated with egg density (Ianora & Santella 1991; Belmonte & Puce 1994; Belmonte et al. 1997). No significant variations between experimental and *in situ* density were detected in our study, because our experimental research was carried out simulating *in situ* conditions. Based on results from our study, it can be concluded that the mean spine length shows a positive relationship with water density and an inverse relationship with it when temperature decreases (Figure 6). Furthermore, as no data on egg density were collected in our study, no clear pattern that could help explain the relationship between spine length, density of the natural environmental medium, egg density and temperature could be established. Therefore, although we agree that external environmental factors modulate egg ontogeny, in our case these characters could have been regulated mainly by maternal inheritance.

Our results also suggest that the external morphology of eggs produced during the cold season was independent of hatching success. The three egg morphotypes identified in our study (EI, EII, EIII) had similar abilities as resting eggs on account of the fact that they all showed low hatching. Several studies have reported variations in the spine length of eggs of *Acartia*, resting eggs being the morphotype with the longest spines (e.g. Marcus 1991; Castro-Longoria 2001; Onoue et al. 2004; Belmonte & Pati 2007). No association between the morphotype with longer spines (EIII) and lower % hatching was found in our study in relation to other eggs also produced during the cold season. Furthermore, all egg morphotypes produced during the cold season were found to have a similar hatching behaviour and no changes in spine length were observed at the end of embryogenesis in the last period of incubation. The hypothesis on the close relationship between egg morphotypes and spine lengths, as observed in *Centropages velificatus* (Oliveira, 1947) (Blades-Eckelbarger & Marcus 1992), should thus be discarded for *A. tonsa* eggs from BBE. In the present study, it was observed that eggs with longer spines (EIII) were in a minority in relation to other morphotypes produced during the cold season. Although we did not have any empirical data on the densities of these eggs, it was observed that it took longer for EIII eggs to settle to the bottom of the experimental devices. Thus, if our findings regarding hatching suggest that external morphology is independent of *A. tonsa*'s ability to develop and hatch, the question that arises is, why is it that *A. tonsa* produces three egg morphotypes with different spine lengths during the cold season in BBE and what could be the ecological potential of this phenomenon? No answer has yet been given. Such behaviour coincides, in fact, with that of females taken from a semi-extensive culture carried out in Limfjord, Denmark (Hansen et al. 2010b) during the cold season. As in our study, Hansen et al. (2010b) reported that single females produced several types of eggs with or without surface spines even within the same egg laying. Taken together, our observations and preliminary results therefore lead us to propose that the different egg types may display specific roles to ensure population permanence through the annual cycle in BBE. In line with this, we observed the presence of EIII eggs suspended in the surface for a long period of time whereas EI and EII eggs were found deposited at the bottom of the experimental devices. It could be assumed that EIII eggs remain suspended longer in the water column, which could be particularly useful in a highly dynamic estuary with permanent resuspension (Belmonte et al. 1997) and this seems to be the case for BBE. The presence of EIII eggs therefore seems to ensure the short-term recruitment of the population during the cold season. The other two morphotypes may behave as the benthic phase, contributing to long-term population recruitment (Grice & Marcus 1981). The presence mainly of EI and EII eggs and the absence of EIII eggs in natural sediments were confirmed by observations in a benthic bank of BBE (Diodato et al. 2006; Berasategui et al. 2013). A delay in hatching (several months) at temperatures > 15°C was in fact reported in *A. tonsa* eggs from the benthic bank in BBE (Berasategui et al. 2013).

Small females and a high production of subitaneous eggs were both observed during the warm season, this being a trend that was also confirmed by SEP values. This strategy indicates a clear tendency to focus energy on reproduction, which is consistent with the typical high abundances of this species during the warm season in BBE (Sabatini 1989; Hoffmeyer 2007). Nevertheless, the maximum values of EP recorded in our study (12 eggs f^{-1} day^{-1}, under 20–23°C and 30–35 of salinities) were relatively low compared to other results reported for female culturing. This could be due to a combination of high temperature and salinity along with low quality of food in BBE during the warm season. Holste & Peck (2006) recommended a temperature of 18 ± 0.5°C and salinities of 14–20 to reach the maximum production of subitaneous eggs (>20 eggs f^{-1} day^{-1}) in an *A. tonsa* culture (Danish Institute for

Fisheries and Marine Research, Charlottenlund, Denmark; *A. tonsa* culture of more than 70 generations). This study, as well as others by Båmstedt & Skjoldahl (1980), Tester & Turner (1990) and Hansen et al. (2010a), was carried out after an acclimatization period and with a high quality of food. The reproductive responses of the females analysed were thus found to be more favourable than the responses of wild females incubated under *in situ* conditions and without acclimation, as was done in our study. As to quantity–quality of food, a high contribution of detritus in the natural diet of *A. tonsa* during the warm season has been reported in BBE (Diodato & Hoffmeyer 2008). A high concentration of suspended particulates in the water column could limit the egg production rate of copepods, because detritus hinders prey capture (Sherk et al. 1976; White & Dagg 1989) or because detritus intake reduces food quality (Gasparini et al. 1999; Calliari et al. 2004).

Results from our study demonstrate that in BBE *A. tonsa* has a marked seasonality in its reproductive pattern, which ensures its permanence in the water column throughout the year. They also lead us to conclude that *A. tonsa*'s production of delayed hatching eggs as well as its great tolerance to low temperatures and high salinities suggest that its population could have interesting applications in aquaculture.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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