NOTE
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MORPHOLOGICAL TYPES AND SEASONAL VARIATION IN EGGS OF ZOOPLANKTON SPECIES FROM BOTTOM SEDIMENTS IN BAHÍA BLANCA ESTUARY, ARGENTINA*

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Resting egg production is a survival mechanism in a wide variety of marine, estuarine, and fresh water zooplankton species (including copepods, rotifers, and cladocerans) against adverse environmental conditions (Kasahara *et al*., 1974; Kasahara *et al*., 1975; Uye, 1985; Hagiwara & Hino, 1990; Lindley, 1990; Marcus, 1990; Marcus *et al*., 1994; Katajisto, 1996; Moscatello *et al*., 2004; Katajisto, 2006). Egg accumulation in the upper layer of the bottom sediments is fundamental to the resurgence of the populations of these species when conditions become more favourable (Marcus, 1996). Rotifers, such as *Synchaeta* sp. (Barría de Cao, pers. comm.), are commonly found in Bahía Blanca Estuary, Argentina (38º 30'-39º 25' S; 61º 15'-63º 00' W). The presence of this microzooplankton species in the estuary is highest from winter to the beginning of spring (Barría de Cao, pers. comm.). In addition, the most abundant planktonic copepods in this area are *Acartia tonsa* Dana and *Eurytemora americana* Williams. Whereas the former is the native key species which is present throughout the year evidencing peaks in summer, the latter is an invader species that evidences only a short winter-spring pulse (Hoffmeyer, 2004). The three abovementioned species produce resting eggs which are commonly found in the bottom sediments of Bahía Blanca Estuary (Hoffmeyer *et al*., 2003**; Molina, 2005; Barría de Cao, pers. comm.).

Resting eggs belonging to the rotifer *Synchaeta* sp. are considered as truly diapausal (Moscatello *et al*., 2004). On the other hand, certain studies on copepods reviewed by Marcus (1996) consider *A. tonsa* eggs as diapausal. In addition, Castro-Longoria (2001) by incubating *A. tonsa* females from Southampton, United Kingdom, concluded that eggs with intermediate spines could be considered to be diapause in character. However, and according to recent studies conducted in the northernregion of the Baltic Sea by Katajisto *et al*. (1998) and Katajisto (2006), the egg type produced by *A. tonsa* is described as intermediate between subitaneous and diapause, resembling the so-called "delayed -hatching" egg type. Berasategui's findings (2005) regarding the incubated eggs of *A. tonsa* females exposed to different experimental temperature regimes in Bahía Blanca, showed a similar behaviour. In the case of *E. americana* eggs, Marcus *et al.* (1994) classed them - in general - as resting eggs. However, and taking into account their short cycle as active phase in the plankton of this estuary (June to October) as well as their phylogenetic relationship with *Eurytemora affinis* Poppe, it could be suggested that, within resting eggs, they belong to the diapause type (Ban, 1992; Ban & Minoda, 1991; Katajisto, 1996; Katajisto, 2006).

Morphological data regarding subitaneous and resting eggs of *A. tonsa* as well as subitaneous eggs of *E. americana* found in Bahía Blanca Estuary have been obtained by optical microscopy and scanning electron microscopy (SEM) (Hoffmeyer *et al*., 2003). These authors described three types of subitaneous eggs of *A. tonsa* (type 1, 2, and 3) taking into account their diameter and particularly the length of spines. They also described subitaneous eggs of *E. americana* which showed a smooth surface under light microscope and a rough surface similar to brain convolutions under SEM (Hoffmeyer *et al.,* 2003). The resuspension of resting eggs of *A. tonsa* -which, according to Hoffmeyer *et al*. (2003) are similar to subitaneous eggs type 1 and 2- from the bottom to undergo hatching has been reported by Molina (2005). According to this author, this phenomenon occurs mainly as a result of the hydrodynamic effect of waves.

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Though in other parts of the world research in this field has greatly advanced after several decades of study, to our knowledge, our research is carried out for the first time in Argentina. Our findings, which are of preliminary nature, constitute the beginning of a line of research that focuses on the spatial and seasonal distribution of this type of eggs and the role of resting egg production as a survival mechanism in Bahía Blanca Estuary plankton.

In the present work we studied eggs isolated from samples of bottom sediments obtained from two sites which had been chosen for different research plans. One site is Cuatreros Port where our study took one year and the other site is Ing. White Port where our study took only one month. Both sites are located within the inner part of Bahía Blanca Estuary (Fig. 1). No attempt of making a comparative analysis between the two sites was made in the present study.

The aims of our work were to describe the morphological types of eggs found in bottom sediments (namely type A, B, C, D, E, F, G and H); to try to identify to which species the eggs found belong; to corroborate the identity of some of them by means of incubations; and finally, to determine either the spatial or seasonal presence of morphological types according to the sampling site.

Sediment samples were collected from Cuatreros Port on a monthly basis from January to December 2003. Bottom silt-clay sediment samples were extracted manually using an Eckman dredge. The first centimeter of the upper surface of the sediment was removed with a spatula and was generally placed in 4 cm-Petri capsules although larger receptacles were used on certain occasions to this end. We could not therefore carry out the quantitative analysis of eggs in the samples. Samples from Ing. White Port were collected from the area close to the industrial zone in April 2004. In this case, original and replica mud sediment samples were collected from three stations (1, 2 and 3) from intertidal and subtidal areas. In the former, samples were collected at low tide from the coast line following the abovementioned procedure. In the subtidal areas, samples were collected using an Eckman dredge from aboard a motor boat and subsequently following the same procedure as that in Cuatreros Port. In the laboratory, all samples from

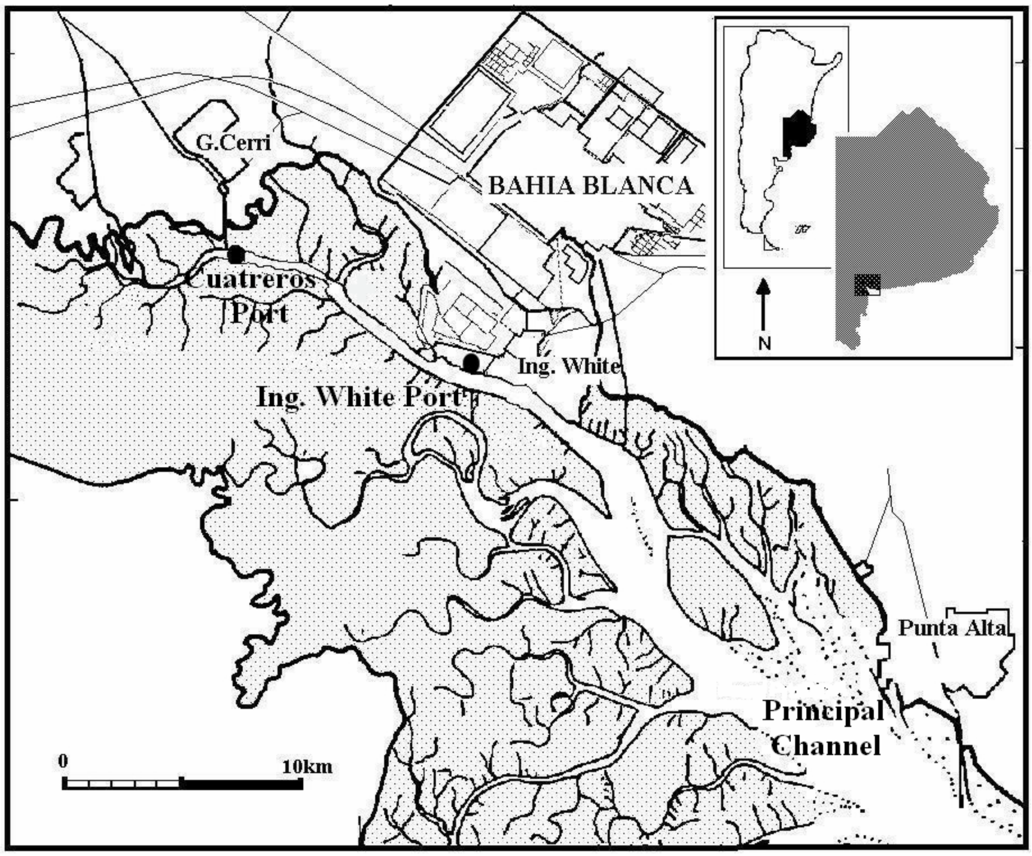

Fig. 1. Location of the sampling zones (Cuatreros and Ing. White Ports, Bahía Blanca Estuary, Argentina).

both sites were processed following the same procedure. They were refrigerated at 4ºC, hydrated with filtered seawater (vacuum using GF/C membranes), and they were homogenized with an ultrasonic Bandelin Sonorex TK52 cleaner for 5 min. to facilitate disaggregation of the sludge. Samples were subsequently sieved through two metallic mesh sizes -Nº 80 and 270 (180 µm and 53 µm, respectively) - under continuous washing with filtered seawater. The remaining sediment was treated using the sugar flotation method of Onbé (1978). The supernatant was washed using a 30 µm mesh filter in order to retain the eggs. A stereomicroscope Wild M 5, and a light microscope C. Zeiss-Jena Laboval were used to measure the diameter of the eggs as well as to briefly describe their morphology taking into account the shape and external surface ornamentation. Microphotographs of eggs were taken with a Nikon Coolpix 4300 digital camera attached to the light microscope.

Incubations with eggs - supposedly of *A. tonsa* - collected from Cuatreros Port and Ing. White Port bottom sediments samples were sometimes carried out to corroborate their taxonomic identity as well as to determine their character of resting eggs. This species is dominant in the mesozooplankton and its population dynamics in this environment has been thoroughly studied (Sabatini, 1989; Hoffmeyer, 2004). Egg incubations from both sites were carried out in 100 ml crystallizers with 0.45 µm filtered seawater at 18 ± 1ºC. This temperature is similar to the optimal temperatures found *in situ* (between 17 and 20 ºC) at which this species reaches its maximal population density (Hoffmeyer pers. comm.). In the case of Cuatreros Port, incubation was carried out only with the eggs from the sample collected in July 2003 as it was the one that evidenced the presence of the three morphological types of *A. tonsa* eggs (Hoffmeyer *et al*., 2003). They correspond to the egg types B, C, and D described below. In this case, an11-day incubation was started with only 2 h cooling. This cooling period, which is short, cannot be considered a true refractory phase. Incubation with the eggs type F collected from Ing. White Port was carried out during 9 days with a previous refractory phase of 3 and a half months at 6ºC in an isothermal room.

Five morphological types of eggs (type A, B, C, D and E) were found in the sediments from Cuatreros Port. The features typifying each one of them are as follows:

Type A: ovoid eggs with very marked ornamentation, resembling small hooks which are visible on the surface under light microscope (400X). Average dimensions: 79.32 µm wide (SD = 0.37 µm) and 115.54 µm long (SD = 0.77 µm) (n = 10) (Fig. 2a). These characteristics agree with those mentioned by Moscatello *et al*. (2004) for diapausal eggs of the rotifer species *Synchaeta* sp. They described two morphological types of eggs, namely, smooth subitaneous eggs and diapausal eggs with a rough surface and curved spines resembling small hooks all over their surface. The majority of the eggs belonging to this species were found in the samples collected in November whereas a few of them were found in December, January, March, June, and August (Table 1). The absence of the rotifer *Synchaeta* sp. in the plankton and its presence in the bottom sediments particularly during the warm months agree with observations reported by Moscatello *et al*. (2004) on the inverse correlation between the presence of resting eggs in the sediments and the active stages in the water column on the coast of Salento, Italy. Our findings could be explained taking into account the short planktonic pulse of this species that occurred in Bahía Blanca Estuary between June and October in 2003 (Barría de Cao, pers. comm.).

Type B: spherical eggs, with smooth chorion or with short spines visible under light microscope (400X). Average diameter: 85 µm (SD = 0) (n = 6) (Fig. 2b). According to Hoffmeyer *et al*. (2003), they display a similar aspect to that of subitaneous eggs type 1 of *A. tonsa*, (93.39 µm in diameter, they are smooth or with tinny spines 1-1.5 µm in length) although their size is smaller. In addition, eggs type B coincide in morphology but not in size with the subitaneous eggs described by Castro-Longoria (2001) (average diameter: 76.54 µm ± 2.9 µm). As for the incubation experiment carried out, all eggs type B – whose identity corresponded to *A. tonsa* according to the taxonomic determination of nauplius – hatched during 11 days. This fact as well as the egg appearance in several bottom samples collected all along the year may confirm that they are resting eggs of the "delayed-hatching" type (Katajisto, 2006). Experimental results obtained from incubations of *A. tonsa* females carried out all along the year by Berasategui (2005) confirm this behaviour resulting from the combined effect of the female previous history as well as of low temperatures on the hatching of recently produced eggs. These type B eggs were found in bottom samples collected in January, June, and July 2003 (Table 1). The eggs found in January appeared to be non-viable because of the embryo displacement to one of the poles. In contrast, the eggs collected in June and July probably gave rise to the recruitment of *A. tonsa* for the spring peak.

Type C: spherical eggs with intermediate spines visible under stereomicroscope (400X). Average diameter including spines: 93.5µm (SD = 0.84 µm) (n = 7). They have a similar aspect to that of the subitaneous eggs type 2 of *A. tonsa* with a diameter of 97.67 µm, intermediate spines of 8.5-17 µm in length (Hoffmeyer *et al.,* 2003). No hatching occurred during the incubations as probably the eggs were non-viable although they seemed to belong to the subitaneous class. They appeared only in July (Table 1) which coincides with the highest number of this egg type spawned by females during the same month in laboratory experiments (Berasategui, 2005).

Type D: spherical eggs with long spines clearly visible under stereomicroscope (400X). Average diameter including spines: 119 µm (SD = 0) (n = 2). Similar to the subitaneous eggs type 3 of *A. tonsa* described by Hoffmeyer *et al.* (2003), with a diameter of 97.51 µm and large spines of approximately 22.24 µm in length. After incubation, these subitaneous eggs did not hatch, thus showing no viability. Based on female incubation experimental results, Berasategui (2005) observed that these subitaneous eggs are produced only in winter in very low densities, and reported a faster eclosion than that evidenced by the other types of eggs. This behaviour may explain the very low density in bottom samples detected in this study, a phenomenon which also agrees with Molina´s (2005) observation on the total absence of these eggs. The abovementioned characteristics could be indicative of the reason why these eggs were only found in the samples corresponding to July (Table 1).

Type E: spherical eggs with small inclined scales covering all the surface and showing a particular dotted pattern on the chorion under light microscope (400X). Average diameter: 88.74 µm (SD = 0.61 µm) (n = 5). They may be preliminarily considered as eggs of *E. americana* because they resemble – in appearance – the subitaneous eggs of this species (Hoffmeyer *et al.*, 2003). According to these authors, these eggs laid by incubated females evidence a smooth surface under light microscope and a rough surface similar to brain convolutions under SEM. They were observed only in January and March in coincidence with the absence of their population in the water column (Table 1).

The morphological types of eggs from the sediments at Ing. White Port are the following:

Type F: eggs with an apparently smooth surface with adherents in some cases. Under light microscope (400X) a thick wall is observed, which sometimes has sporadic tinny spines. Average diameter: 91.08 µm (SD = 0.61 µm) (n = 31) (Fig. 3a). As to the general aspect, these eggs are similar to the subitaneous eggs type 1 of *A. tonsa* (Hoffmeyer *et al.*, 2003) although there are some differences in size and in the wall thickness. On account of the fact that the sediment in the intertidal zone contained a very low number of whole eggs, those eggs belonging to replica samples from the subtidal zone were used for incubation experiments. After 9 days, all eggs hatched. The taxonomic determination of nauplii indicated that they belonged to *A. tonsa.*

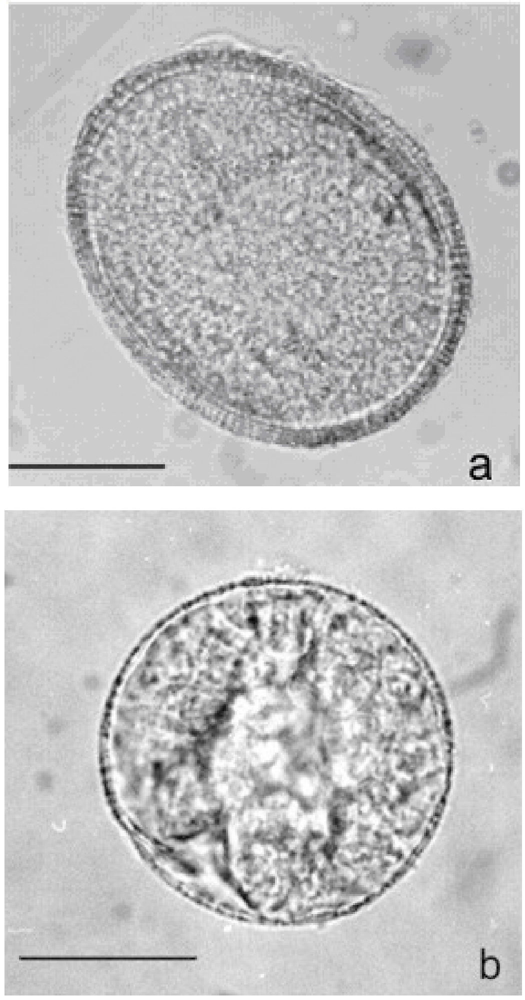

Fig. 2. Resting eggs found in the upper layer of bottom sediments at Cuatreros Port. (a: *Synchaeta* sp.; b: *Acartia tonsa*). Bar scale: 50 µm.

Type G: eggs with smooth chorion and a diameter ranging between 60 and 65µm. They were found in masses, with some eggs adhered to the remains of females. As the presence of these eggs was registered only in the intertidal area (Table 2), they seem to belong to the harpacticoid benthic copepod *Nannopus palustris* (Hoffmeyer, 2004). At the moment of carrying out the sampling, this species was more abundant in the meiobenthos than as an adventitious form in the plankton of the intertidal zone (Hoffmeyer & Parodi, pers. comm.). According to the conditions under which these eggs were found in the samples, it can be inferred that they were non-viable eggs.

Table 1. Seasonal occurrence of the different morphological types of eggs in bottom sediments of Cuatreros Port, Bahía Blanca estuary, along the year 2003. *: presence of eggs in samples.

Morphological types	J	F	M	A	M	J	J	A	S	O	N	D
A	*		*			*		*			*	*
B	*					*	*					
C							*					
D							*					
E	*		*									

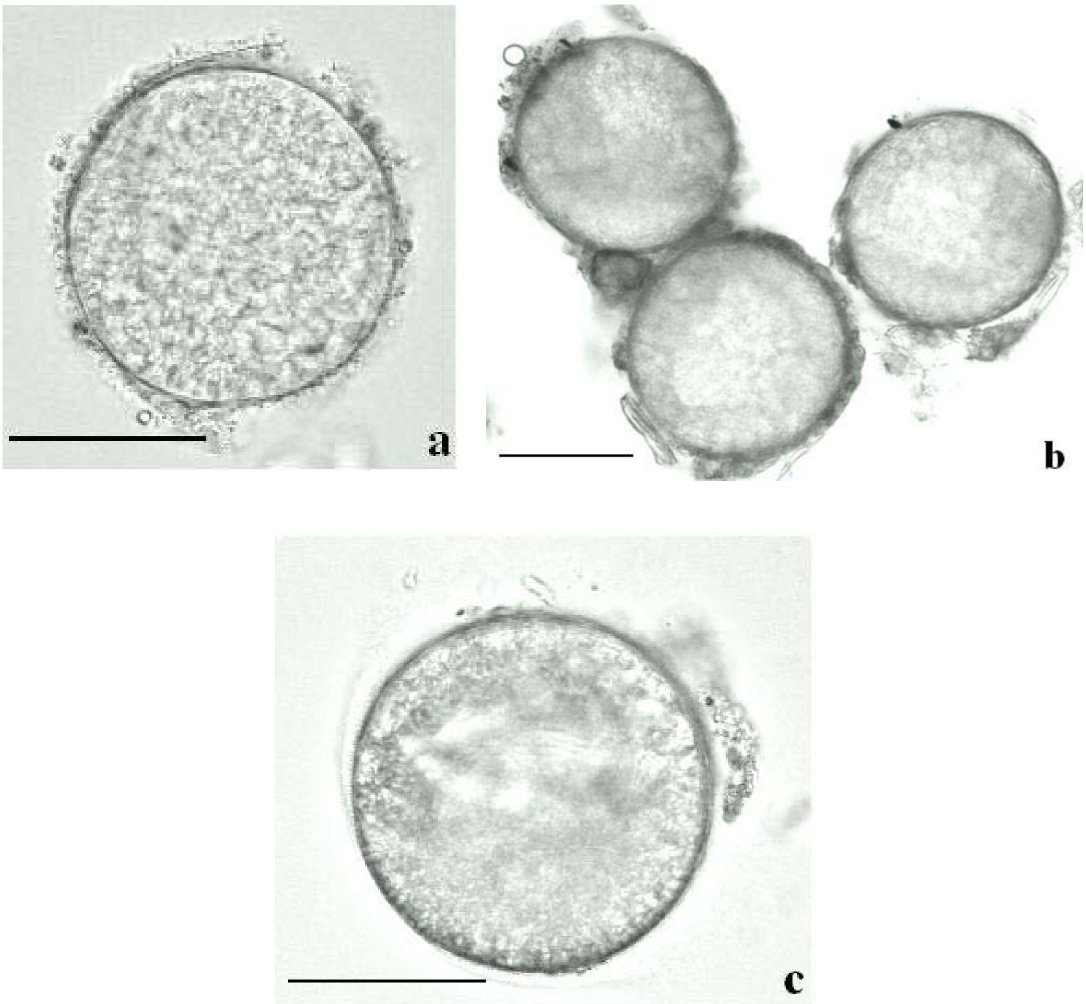

Fig. 3. Copepod eggs found in the upper layer of bottom sediments at Ing. White Port. (a: *Acartia tonsa*, b: *Eurytemora americana* (egg mass), c: *Eurytemora americana*). Bar scale: 50 µm.

Type H: eggs with smooth chorion and a characteristic dotted pattern similar to brain convolutions under light microscope (400X). Their diameter is approximately 90.1 µm. They were found in masses with some adherences of the remains of the ovigerous sac (Fig. 3b) and they were similar to those described as subitaneous eggs of *E. americana* by Hoffmeyer *et al.,* 2003. It has been reported -in relation to other species of the same genus (*E. affinis*)- that under natural conditions females carry the eggs, which can be either subitaneous or diapausal, in an ovigerous sac depending on the particular time of the year and the environmental conditions (Ban & Minoda, 1991). According to these authors, these eggs are liberated individually as they reach embryonic maturity. Subitaneous eggs undergo immediate hatching whereas diapausal eggs rest in the sediment for an extended refractory period until more favourable environmental conditions prevail. They must be then resuspended to hatch (Ban, 1992). As in our study, eggs type H were found in masses rather than individually in the sediment, it can be hypothesized that they could be either diapausal or subitaneous eggs with no complete embryonic development. In view of this, they were non-viable eggs of *E. americana.*

Summing up, of the 8 morphological types of eggs found in this study, only those of the rotifer *Synchaeta* sp. in Cuatreros Port and the copepod *A. tonsa* in the two sampling sites were confirmed to be respectively diapausal and "delayed -hatching". Both classes were corroborated by comparison with reports by Moscatello *et al.* (2004) in the former case and by incubation experiments in the latter case. The other identified eggs from Ing.White Port belong to *E. americana* and *Nannopus palustris* which appeared to be non-viable eggs trapped in the sediment.

As regards the seasonal variation of the eggs found in the sediments from Cuatreros Port and the active forms of the populations of *Synchaeta* sp. and *A. tonsa*, our study confirms the presence of eggs in the sediment either in periods of time during which no active forms of these species have been previously reported in the plankton (*Synchaeta* sp. between November to May, according to Barría de Cao, per. comm.) or in periods of time during which only some active forms of these species (*A. tonsa* during June-July according to Hoffmeyer, 2004) have been reported at very low densities.

Concerning the spatial variation at Ing.White Port, the differences in the presence of eggs across the sampling areas may be due to sediment modifications produced by anthropic impact, particularly at station 2 of the intertidal area where industrial effluents are discharged (Table 2). Eggs type F evidenced the highest frequency of occurrence with respect to eggs type G and H in both tidal zones. This area is characterized by the presence of large-grained sediment with large interstitial spaces, which may prevent either the contention or adhesion of resting eggs that are easily washed away by the movement of tides.

Table 2. Spatial occurrence of different morphological types of eggs in bottom sediments at intertidal and subtidal zones of Ing. White Port, Bahía Blanca estuary. *: presence of eggs in samples. *̲: eggs in masses.

Morphological types	Intertidal			Subtidal		
	1	2	3	1	2	3
F	*		*	*	*	*
G	*̲		*̲			
H						*̲

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References

Ban, S. 1992. Seasonal distribution, abundance and viability of diapause eggs of *Eurytemora affinis* (Copepoda: Calanoida) in the sediment of Lake Ohnuma, Hokkaido. Bull. Plankt. Soc. Japan, 39(1):41-48.

Ban, S. & Minoda, T. 1991. The effect of temperature on the development and hatching of diapause and subitaneous eggs in *Eurytemora affinis* (Copepoda: Calanoida) in Lake Ohnuma, Hokkaido, Japan. In: Uye, Nishida & Ho eds. Proceedings of the Fourth International Conference on Copepoda. Bull. Plankt. Soc. Japan, (Spec., Vol).:299-308.

Castro-Longoria, E. 2001. Comparative observations on the external morphology of subitaneous and diapause eggs of *Acartia* species from Southampton water. Crustaceana, 74(3):225-236.

Hagiwara, A. & Hino, A. 1990. Feeding history and hatching of resting eggs in the marine rotifer *Brachionus plicatilis.* Nippon Suis. Gakk., 56(12):1865-1971.

Hoffmeyer, M. S. 1994. Seasonal succession of Copepoda in the Bahía Blanca Estuary. In: Ferrari, F. D. & Bradley, B. P. eds. Ecology and morphology of copepods. Hydrobiologia, 292/293:303-308.

Hoffmeyer, M. S. 2004. Decadal change in zooplankton seasonal succession in the Bahía Blanca Estuary, Argentina, following introduction of two zooplankton species. J. Plankt. Res., 26(2):1-9.

Kasahara, S.; Uye, S. & Onbé T. 1974. Calanoid copepod eggs in sea bottom muds. Mar. Biol., 26: 167-171.

Kasahara, S.; Uye S. & Onbé T. 1975. Calanoid copepod eggs in sea-bottom muds. II. Seasonal cycles of abundance in the populations of several species of copepods and their eggs in the inland seas of Japan. Mar. Biol., 25-29.

Katajisto, T. 1996. Copepod eggs survive a decade in the sediments of the Baltic Sea. Hydrobiologia, 320:153-159.

Katajisto, T.; Viitasalo, M. & Koski, M. 1998. Seasonal occurrence and hatching of calanoid copepods in sediemnts of the northern Baltic Sea. Mar. Ecol. Prog. Ser., 163:133-143.

Lindley, J. A. 1990. Distribution of overwintering calanoid copepod eggs in the sea-bed sediments around southern Britain. Mar. Biol., 104:209-217.

Marcus, N. H. 1990. Calanoid copepod, cladoceran, and rotifer eggs in sea-bottom sediments of northern Californian coastal waters: identification, occurrence and hatching. Mar. Biol., 105:413-418.

Marcus, N. H. 1996. Ecological and evolutionary significance of resting eggs in marine copepods: past, present, and future studies. Hydrobiologia, 320:141-152.

Marcus, N. H.; Lutz, R., Burnett, W. & Cable, P. 1994. Age viability and vertical distribution of zooplankton resting eggs from an anoxic basin: Evidence of an egg bank. Limnol. & Oceanogr., 39(1):154-158.

Moscatello, S.; Rubino, F.; Saracino, O. D.; Fanelli, G.; Belmonte, G. & Boero, F. 2004. Plankton biodiversity around the Salento Peninsula (South East Italy): an integrated water/sediment approach. In: Ros, J. D.; Packard, T. T.; Pili, J. M.; Pretus, J. L. & Blasco, D. eds. Biological oceanography at the turn of the Millenium. Sci. Mar., 68 (Suppl.1):85-102.

Onbé, T. 1978. Sugar flotation method for sorting the resting eggs of marine cladocerans and copepods from sea-bottom sediment. Bull. jap. Soc. sci. Fish., 44:1411.

Sabatini, M. E. 1989. Ciclo anual del copépodo *Acartia tonsa* (Dana, 1849) en la zona interna de la Bahía Blanca (Pcia. de Buenos Aires, Argentina). Sci. Mar., 53:847-856.

Uye, S. 1985. Resting egg production as a life history strategy of marine planktonic copepods. Bull. Mar. Sci., 37:440-449.

Sources of Unpublished Material

Berasategui, A. A. 2005. Efecto de la temperatura experimental sobre la producción y eclosión de huevos de *Acartia tonsa* (Copepoda, Calanoida). Tesis de grado. Bahía Blanca. Argentina. Universidad Nacional del Sur. 28 p.

Katajisto, T. 2006. Benthic resting eggs in the life cycles of calanoid copepods in the northern Baltic Sea. Ph.D Thesis. Helsinki, Walter and Andrée de Nottbeck Found.Scient. Reports, no. 29. 159p.

Molina, L. 2005. Resuspensión de huevos de copépodos y sedimentos en una planicie de marea de Puerto Cuatreros, estuario de Bahía Blanca. Tesis de grado. Bahía Blanca. Argentina. Universidad Nacional del Sur. 35 p.

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