Patterns in rotifer diapausing egg banks: Density and viability

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Abstract

Here we present a quantitative study on the density, age and viability of the diapausing egg banks of the rotifer species complex *Brachionus plicatilis* in the sediments of 15 water bodies from Eastern Spain. Sampled ponds, located in coastal and inland areas, varied in salinity and ranged in size, depth and permanence. By identifying 'hatched', 'deteriorated' and 'viable' diapausing eggs in the sediment samples, we estimated production, hatching and deterioration in relation to the habitat properties of each pond. Our results indicate the presence of large numbers of diapausing eggs in the sediments of almost all of the ponds studied (2–115 eggs cm^{-2}). Inland ponds tended to have higher densities than coastal lagoons. The vertical distribution of eggs in the sediments frequently showed a non-decreasing pattern, which suggested a high among-year variation in egg production. Despite maximum age of eggs of 60–80 years, the median age (3–30 years) suggests that rotifer egg banks are young in the studied ponds. Egg senescence is suggested by the declining abundance of 'healthy-looking' eggs with depth. The proportion of 'deteriorated' eggs ranged 75–99% suggesting that deterioration rates in the sediments are high and vary among habitats. Hatching and deterioration rates, as estimated from the counts of 'hatched', 'deteriorated' and 'healthy-looking' eggs in the sediments, largely varied among ponds. An association between hatching and deterioration rates is suggested by our data. This is in agreement with the hypothesis that hatching rates of diapausing eggs depended not only on the risks associated with the water column, as initially expected by the general theory on diapause, but they are also related to the incidence of deterioration processes in the sediment.
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Keywords: Deterioration; Diapausing egg banks; Hatching; Production; Rotifers

1. Introduction

Continental zooplankton usually live in temporary habitats and overcome unfavourable environmental conditions by producing diapausing eggs. These eggs are produced by individuals from the active population in the water column in advance of adverse conditions (i.e., exclusion by interspecific interactions, pond desiccation, extreme physico-chemical conditions). Once produced, diapausing eggs sink and remain in the sediments of ponds and lakes where they are able to persist until favourable conditions resume (Gilbert, 1974; Wiggins et al., 1980). Since diapausing eggs are the only way for these animals to survive, diapausing egg production has been recognized to be a fitness component in temporary populations (Serra et al., 2003). When favourable conditions return, only a fraction of the diapausing eggs in the bank hatches and contributes to active population growth. Another fraction remains viable in the sediments as a persistent egg bank that buffers bad years and spreads the risk of local extinction (for review see Brendonk and De Meester, 2003).

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Despite their importance in the dynamics and evolution of zooplankton populations, diapausing eggs have typically received much less attention than animals in the water column. As a consequence, demographic links between active animals and diapausing stages remain largely unexplored (Cáceres, 1998). However, in recent years, demography of diapausing egg banks and pelagic–benthic coupling in zooplankton dynamics have been increasingly addressed by zooplankton ecologists for two of the main taxa—cladocera and copepoda (e.g., De Stasio, 1989; Cáceres and Hairston, 1998; Marcus and Boero, 1998; Hairston et al., 2000). This is not the case for rotifer diapausing egg banks, which have been poorly studied. A review of the literature on rotifer diapausing egg banks reveals only eight papers published in 50 years. Moreover, almost all studies on diapausing egg banks refer to large, deep freshwater lakes, whereas small, shallow, brackish ponds are completely ignored. Because diapause patterns are expected to correlate with both the ecological regime of zooplankton populations and other life-history characteristics, studies on several types of water bodies, and on all the major zooplankton taxa, are needed in order to achieve an unbiased view of zooplankton diapause.

Among rotifers, the cyclical parthenogen *Brachionus plicatilis* (Müller 1786) is one of the best-known taxa. This taxon usually inhabits temporary environments and its long-term persistence in a habitat depends on the production of diapausing eggs. Recent studies have demonstrated that the *B. plicatilis* taxon is a complex of a still undetermined number of species (Gómez et al., 1995, 2002; Ortells et al., 2000). The species of the *B. plicatilis* complex inhabit and form diapausing egg banks in a wide range of ponds and lakes in Spain (Ortells et al., 2000). These water bodies vary in size, depth, salinity and hydrological pattern (from ephemeral to seasonal ponds, and permanent lakes), this variation representing a spectrum in terms of habitat stability and predictability. This ecological context raises questions about the links between diapausing egg banks and habitat characteristics, and offers a suitable scenario for testing predictions from the general theory on diapause (Cohen, 1966).

In the diapausing egg banks formed by rotifer species belonging to the *B. plicatilis* complex it is feasible to identify eggs in different stages of conservation, as well as 'hatched' eggs (García-Roger et al., 2005). Thus, in these banks, eggs and their remains can be classified as: (1) viable diapausing eggs (identified as 'healthy-looking' diapausing eggs, He), (2) empty shells of diapausing eggs that hatched in the past ('hatched'

diapausing eggs, Ha), and (3) 'deteriorated' eggs (D). The dynamics of these three variables have been recently analyzed by a mathematical model (García-Roger et al., 2006). Accordingly to this model, the summation of the three types of diapausing eggs ($E = $ He + Ha + D) represents the total number of diapausing eggs produced in a pond, when exportation is discounted, and would be indicative of the average quality in the water column. García-Roger et al. (2006) found two ratios among these egg types to be indicative of the importance of hatching (Ha/He) and deterioration processes (D/He) of diapausing eggs while buried in the sediments.

The aim of this study is twofold: (1) to quantify the abundance, age, hatching, and deterioration of rotifer diapausing eggs belonging to the *B. plicatilis* complex in the sediments of a wide range of ponds located in Eastern Spain; and (2) to explore whether properties of these diapausing egg banks were related to independent measurements of habitat features. We studied the differences in diapausing egg bank patterns associated with pond characteristics by using both the observable abundances of the different egg types or their remains in our samples, and meaningful relationships between these abundances, according to the mathematical model proposed by García-Roger et al. (2006).

2. Methods

2.1. Study locations

We sampled 15 brackish ponds and lagoons from two coastal and three inland hydrological areas of Eastern Spain (Fig. 1). Inland ponds are endorheic (i.e., without surface or subsurface outflow; the inflow water being lost by evaporation). The studied water bodies were selected because diapausing egg banks of the *B. plicatilis* rotifer species complex had been previously detected (Ortells et al., 2000). All rotifer populations in these ponds, even those inhabiting permanent ponds, are known to be temporary (Lapesa, 2004). Study ponds differ in size, depth, salinity and hydrological pattern (Table 1), and so they represent a wide ecogeographic range (*sensu* Kratz and Frost, 2000) in which *B. plicatilis* species occur. Ponds were classified according to hydro-period duration and predictability, and following the nomenclature criteria by Brock et al. (2003). Hence, 'permanent' ponds never dry, whereas the other pond categories experience drought periods that differ in frequency and duration. 'Semi-permanent' ponds were those that usually hold some water and may dry out during extreme drought. 'Seasonal' ponds fill and dry cyclically every year. Ponds classified as 'intermittent'
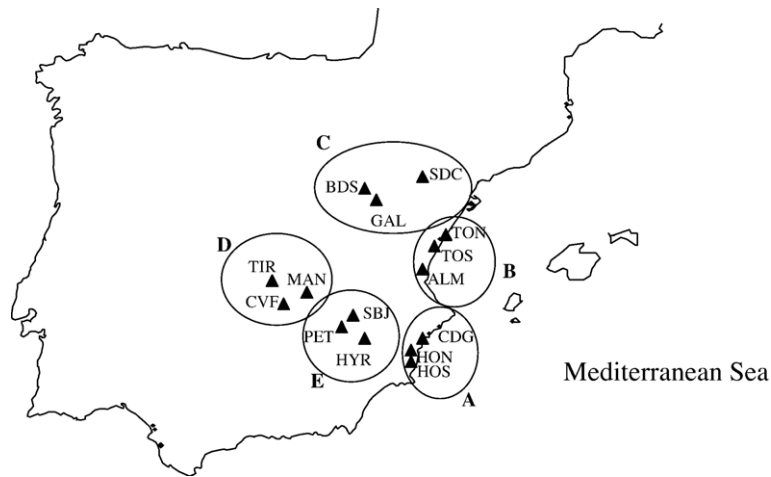
Fig. 1. Map of Spain showing the location of the studied ponds. A. Alicante Coastal Area: CDG, Clot de Galvany; HON, Charca Norte, 'El Hondo de Elche' Natural Park (shortcut: Hondo Norte); HOS, Charca Sur, 'El Hondo de Elche' Natural Park (shortcut: Hondo Sur). B. Castellón Coastal Area: ALM, laguna de Almenara (shortcut: Almenara); TON, Poza Norte, Cabanes-Torreblanca Marsh; TOS, Poza Sur, Cabanes-Torreblanca Marsh. C. Ebro River Basin: BDS, Balsa de Santed; GAL, Laguna de Gallocanta (shortcut: Gallocanta); SDC, Salada de Chiprana. D. Guadiana River Basin: CVF, Laguna del Camino de Villafranca (shortcut: Camino Villafranca); MAN, Laguna de Manjavacas (shortcut: Manjavacas); TIR, Laguna de Tírez (shortcut: Tírez). E. Júcar-Segura River Basin: HYR, Hoya Rasa; PET, Laguna de Pétrola (shortcut: Pétrola); SBJ, Laguna del Salobrejo (shortcut: Salobrejo).

were those that exhibit an alternation between droughts and refills, but less regularly than seasonal ones. Duration of filling in intermittent ponds is highly variable, since water may persist for months or even years. 'Episodic' and 'ephemeral' were drying patterns assigned to those ponds that persist dry for most of the time and only fill after unpredictable rainfall. 'Ephemeral' refers to a higher degree of unpredictability and lower persistence of the water. The assignment of a pond to a category was based on Lapesa (2004) and our own observations. It is worth noting that a continuous variable underlies this rank classification. For statistical

Table 1
Characterization of the study ponds

Hydrological area	Pond	Geographical coordinates	Area (km²)	Depth (m)	Salinity (g l⁻¹)	Hydro-period pattern
Alicante Coastal Area	CDG, Clot de Galvany	38°14.999′N, 0°32.228′W	–	< 1	15.6	Semi-permanent
	HON, Hondo Norte	38°11.384′N, 0°45.140′W	0.125[a]	0.9[a]	10.4	Semi-permanent
	HOS, Hondo Sur	38°10.004′N, 0°44.007′W	0.2[a]	0.8[b]	12.9	Semi-permanent
Castellón Coastal Area	ALM, Almenara	39°45.164′N, 0°11.597′W	0.1[a]	7.5[a]	1.4	Semi-permanent
	TON, Poza Norte	40°08.917′N, 0°10.148′E	0.012[c]	0.8[c]	23.8	Seasonal
	TOS, Poza Sur	40°08.715′N, 0°10.059′E	0.008[c]	1.0[c]	33.0	Seasonal
Ebro River Basin	BDS, Balsa de Santed	41°00.975′N, 1°32.477′W	0.019[d]	< 1	16.5[1]	Episodic
	GAL, Gallocanta	40°58.158′N, 1°31.163′W	14.14[e]	0.5[e]	61.5[1]	Intermittent
	SDC, Salada de Chiprana	41°14.417′N, 0°10.874′W	0.23[f]	5[f]	39.3	Permanent
Guadiana River Basin	CVF, Camino Villafranca	39°25.009′N, 3°16.324′W	1.85[g]	< 1	55.5[2]	Intermittent
	MAN, Manjavacas	39°24.615′N, 2°51.899′W	1.06[f]	0.15[f]	22.9	Semi-permanent
	TIR, Tírez	39°32.631′N, 3°21.114′W	0.62[f]	0.2[f]	77.7	Seasonal
Júcar-Segura River Basin	HYR, Hoya Rasa	38°47.075′N, 1°25.620′W	0.08[f]	< 1	27.7[1]	Seasonal
	PET, Pétrola	38°50.555′N, 1°34.448′W	1.74[f]	0.7[f]	46.0	Semi-permanent
	SBJ, Salobrejo	38°54.765′N, 1°28.275′W	0.36[f]	0.8[f]*	17.4	Semi-permanent

Geographical coordinates were determined using a global positioning system. Data source on surface and average depth of the ponds: a, López (1983); b, Rodrigo et al. (2001); c, Gómez (1996); d, Gracia (1993); e, Pérez et al. (2002); f, Montes and Martino (1987); g, Martí and Del Moral (2002). Reported data on salinity are mean values for three-monthly visits to the ponds during 2001–2002. Pond drying pattern following Brock et al.'s (2003) nomenclature.

[1] Salinity measured in one (winter) visit, since water was not present for the whole sampling period.

[2] Salinity measured in two (winter and spring) visits.

* Maximum depth exceeds 4 m.

analysis, only three general hydrological patterns were considered: (1) long hydro-period, including permanent and semi-permanent ponds; (2) medium hydro-period, ascribed to seasonal ponds; and (3) short hydro-period, for intermittent to ephemeral ponds.

2.2. Sediment sampling

Sampling was conducted in July–August 2001. Sediment samples were obtained from three randomly selected points per pond. Location in the field of the previously selected sampling stations was performed with a global positioning system (Garmin®). Cores were obtained with a piston core sampler (57 mm internal diameter, 60 cm length; Ejkelkamp Agrisearch Equipment). Each core sample was divided in 2-cm slices down to 10 cm. A hydro-pneumatic device (Ejkelkamp Agrisearch Equipment) was used to control sediment discharge and accurately divide the core. All core slices were weighed, placed in 100 ml plastic vessels and conserved at 4 °C in the dark for 2 months (Hagiwara and Hino, 1989).

2.3. Density estimation of diapausing eggs

Sediment fractions of 2 g (wet weight) were taken from the central cylinder of each sediment slice to minimize the risk of egg transfer from upper layers that might occur during sediment core extrusion. These fractions were individually placed in Petri dishes and diluted in 6 g l^{-1} artificial seawater (Instant Ocean®; Aquarium Systems). Following resuspension and deflocculation, samples were filtered consecutively through 200-µm and 30-µm nytal mesh netting. *B. plicatilis* diapausing eggs (length 113–137 µm; width 75–100 µm; Ciros-Pérez et al., 2001) were retained by the 30-µm nytal mesh. No eggs were ever found in the material retained by the 200-µm mesh but, instead, large amounts of bigger-size material were removed (insect

parts, plant seeds, leaves, roots, etc.). The material retained in the 30-µm filter was transferred to a plankton counting chamber and diapausing eggs belonging to *B. plicatilis* were visually identified under a stereomicroscope. On the basis of morphology and/or size, eggs could not be assigned to any particular species of the *B. plicatilis* complex found in the water column of the studied ponds (i.e., *B. plicatilis sensu stricto*, *B. ibericus*, *B. rotundiformis*, *B*. 'Manjavacas', *B*. 'Tíscar' and *B*. 'Almenara'; Ortells et al., 2000).

For the assessment of diapausing egg bank densities, all eggs and their remains in a processed sample were counted. This total number of eggs included diapausing eggs in various stages of conservation as well as already hatched eggs. It is our observation that empty egg shells in *B. plicatilis* persist in the sediment banks and are identifiable in the samples. Total diapausing eggs (E) are considered to be a measurement of diapausing egg production through time, and thus represent the size of the egg bank produced throughout several seasons depending on sedimentation rates. Notice that this is diapausing egg net production, from which egg losses due to predation in the water column or exportation are discounted.

Total diapausing eggs were classified in the following types following García-Roger et al. (2005):

(1) 'Healthy-looking' diapausing eggs (He): eggs that looked good in appearance (Fig. 2A). It is known that almost all hatchlings come from these eggs (García-Roger et al., 2005), so they were assumed to represent the fraction of viable eggs in the banks.

(2) 'Hatched' diapausing eggs (Ha): empty eggs showing the typical opening (i.e., operculum) through which the fully-formed rotifers leave the shell at the hatching moment (Fig. 2B). The finding of a 'hatched' egg indicates that a newborn hatched at some time in the past.
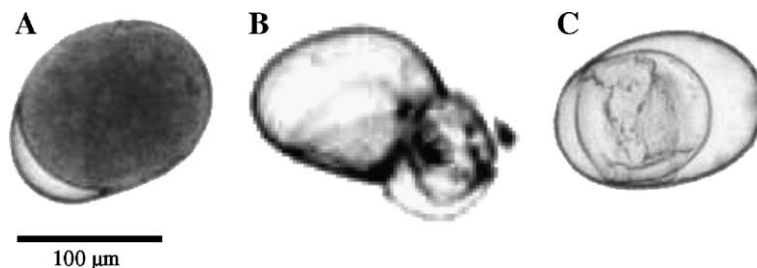

100 µm

Fig. 2. Microphotographs of the different diapausing egg types of *B. plicatilis* found in the sediments of the studied ponds. (A) 'Healthy-looking' diapausing egg. (B) 'Hatched' (i.e., opened via operculum) diapausing egg. (C) 'Deteriorated' egg. All photographs are oriented in such a way that the operculum is on the right side.

(3) 'Deteriorated' diapausing eggs (D): eggs showing embryo reduction, or fractures other than opened operculum (Fig. 2C). These eggs are nonviable.

Counts of each egg type in the 2-g sediment samples were converted to density estimates, expressed as the number of eggs per cubic centimeter of sediment. These densities were also used to estimate diapausing egg density per unit area by integrating over the upper 10 cm of each core.

2.4. Sediment dating and diapausing egg age estimation

The upper 10 cm of sediment from six of our studied ponds (Clot de Galvany, Hondo Sur, Poza Sur, Salada de Chiprana, Camino de Villafranca and Salobrejo) were dated with ^{210}Pb and ^{137}Cs (Krishnaswamy et al., 1971; Ritchie and McHenry, 1990) by G. Benoit at Yale School of Forestry and Environmental Studies (Yale University, CT, USA). Additionally, sedimentation rate for Gallocanta (0.31 ± 0.04 cm year^{-1}) was also available from the literature (Rodó et al., 2002). Sediment dating in this latter study was also performed by ^{210}Pb and validated with ^{226}Ra and ^{137}Cs. Sedimentation rates allowed us to convert depth layers into time and thus, to estimate the age of 'healthy-looking' diapausing eggs at different sediment depths.

2.5. Statistical analyses

Differences in egg bank size (E) were analyzed by means of a generalized linear model (GLM) based on egg counts for each sampling point ($n = 45$). We assumed Poisson distribution for data and log as link function. This model assessed for variation due to location (coastal vs. inland), pond within location, hydro-period pattern and sediment depth. Differences in the ratio of 'healthy-looking' to 'hatched' (He/Ha) and to 'deteriorated' (He/D) diapausing eggs were analyzed by means of GLMs for binary data assuming Binomial distribution for data and logit as link function. It should be noted that ratios studied in this paper are the inverse of ratios in the model by García-Roger et al. (2006) because 'healthy-looking' (He) eggs were not detected in some of the ponds studied. This analysis was restricted to values from the upper (0–2 cm) sediment layer, where egg bank composition is assumed to better describe major processes affecting diapausing eggs in the sediment (García-Roger et al., 2006). These GLMs used the same factors that the GLM for the egg bank size

(E), but excluding sediment depth. The goodness of fit of all models was assessed by the relative change in deviance with respect to the deviance of their respective null models. Interaction terms among factors were included in the models only when improving the fitting to the null models (Johnson and Omland, 2004). All generalized linear models were performed using the *glm* function of *R* 2.1.0 statistical software (Ihaka and Gentleman, 1996).

To explore the relationship within the recorded set of diapausing egg bank properties (egg bank abundance and composition, see below) and habitat features, we performed a principal component analysis (PCA) using SPSS v. 12 (SPSS Inc., Chicago, USA). Variables involved in PCA belong to two categories: (1) habitat properties (i.e., location, hydro-period pattern and log-transformed values for area, depth and salinity of the studied ponds); and (2) abundances of the different types of diapausing eggs studied (i.e., log-transformed values of He, Ha and D). From the latter measurements, relationships involving meaningful parameters (E, Ha/He and D/He, see above) can be derived by inspecting positive and negative linear associations of the measurements. For instance, any axis from the PCA could be explained in part by an association such as log(Ha)–log(He). Then, a high log (Ha)–log(He) implies high log (Ha/He), and then high Ha/He. As suggested from García-Roger et al. (2006), this analysis was restricted to values from the upper (0–2 cm) sediment layer.

3. Results

3.1. Diapausing egg bank characterization

Total egg bank (i.e., 'healthy-looking', 'deteriorated' and 'hatched' diapausing eggs), integrated for the upper 10 cm of sediment and averaged for the three sampling stations, ranged between 4.70 eggs cm^{-2} in Hondo Norte to 26,000 eggs cm^{-2} in Camino de Villafranca. The maximum density in a sampling station (36,400 eggs cm^{-2}) was recorded in this latter pond. Fig. 3 shows the vertical distribution of total diapausing eggs in the sediments of each pond. Eggs and/or their remains were found in all of the sampling stations in all ponds, but not at all depths. The egg bank for the species of the *B. plicatilis* complex extended down at least 8 cm into the sediment of all the studied ponds. Large differences were observed among ponds and among depths within a pond, the latter implying a variety of patterns in diapausing egg distribution. Some ponds (7 of 15) were characterized by a decreasing pattern in total egg
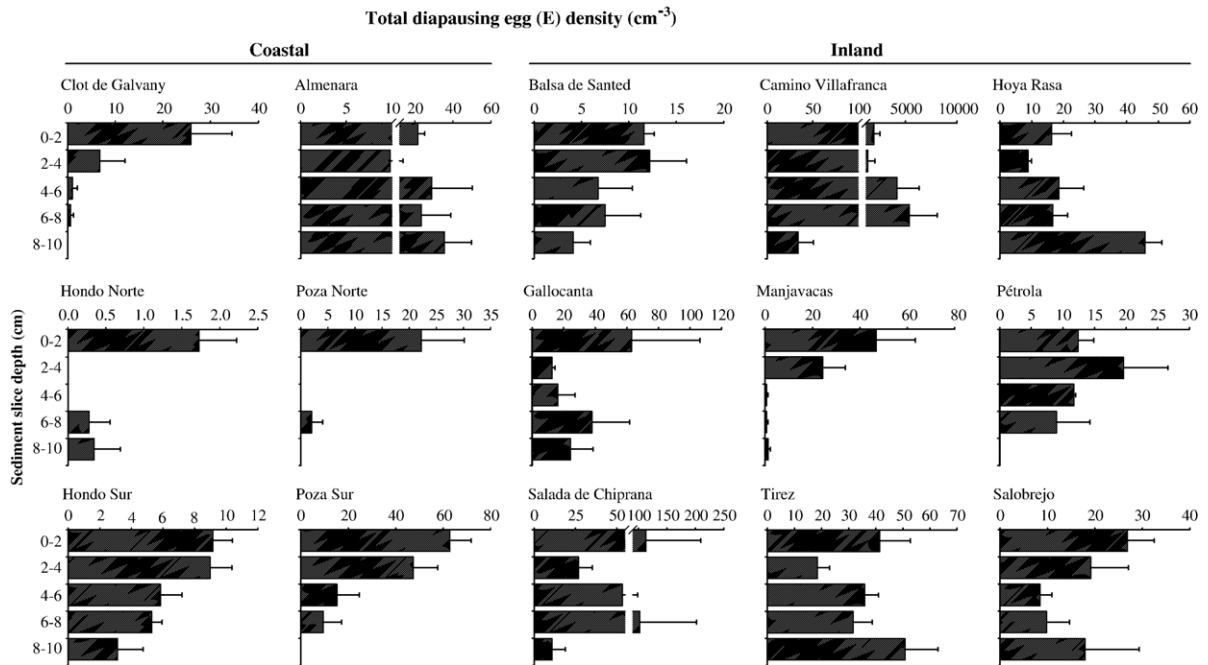
Fig. 3. Density of total diapausing eggs of the *B. plicatilis* species complex at different depths in the sediments of the studied ponds. Data for each sediment layer are means for three sampling stations + 1 S.E. Note the different scales on the *x*-axes.

bank with depth, sometimes leading to extinction. Some other ponds (8 of 15) were characterized by the presence of a subsurface peak of density.

GLM on total diapausing egg counts showed statistically significant differences in egg bank size for all the effects studied (Table 2). As it can be observed in Fig. 4, inland pond sediment had on average more diapausing eggs than coastal (3107 ± 1836 eggs cm^{-2} in inland vs. 115 ± 29 eggs cm^{-2} in coastal). Notice that the

larger variance observed among inland ponds is particularly due to the huge amount of diapausing eggs in Camino de Villafranca. If Camino de Villafranca records are dropped from the analysis, inland ponds still exhibit higher numbers of total diapausing eggs than coastal lagoons. The hydro-period pattern of the ponds also showed significant effects on the size of their diapausing egg banks. Ponds exhibiting short hydro-periods (i.e., intermittent, episodic and ephemeral

Table 2
Summary of implemented generalized linear models

Source of variation	Total egg counts [a]			Ratio He/D [b]			Ratio He/Ha [c]		
	df	Deviance	p	df	Deviance	p	df	Deviance	p
Location	1	10,968	< 0.001	1	207.29	< 0.001	1	93.42	< 0.001
Hydro-period	2	17,566	< 0.001	2	77.48	< 0.001	2	69.36	< 0.001
Depth	4	8697	< 0.001	–	–	–	–	–	–
Location × Hydro-period	1	128	< 0.001	1	1.16	0.28	1	0.49	0.48
Location × Depth	4	806	< 0.001	–	–	–	–	–	–
Hydro-period × Depth	8	1889	< 0.001	–	–	–	–	–	–
Location × Hydro-period × Depth	4	683	< 0.001	–	–	–	–	–	–
Pond (Location × Hydro-period)	10	27508	< 0.001	10	202.01	< 0.001	10	156.54	< 0.001
Pond (Location × Hydro-period) × Depth	39	1776	< 0.001	–	–	–	–	–	–

Notice that the analysis of the He/D and He/He ratios are restricted to the upper sediment layer studied (0–2 cm).
[a] Model: Poisson, link function: log, % Deviance: 84.2.
[b] Model: Binomial, link function: logit, % Deviance: 81.4.
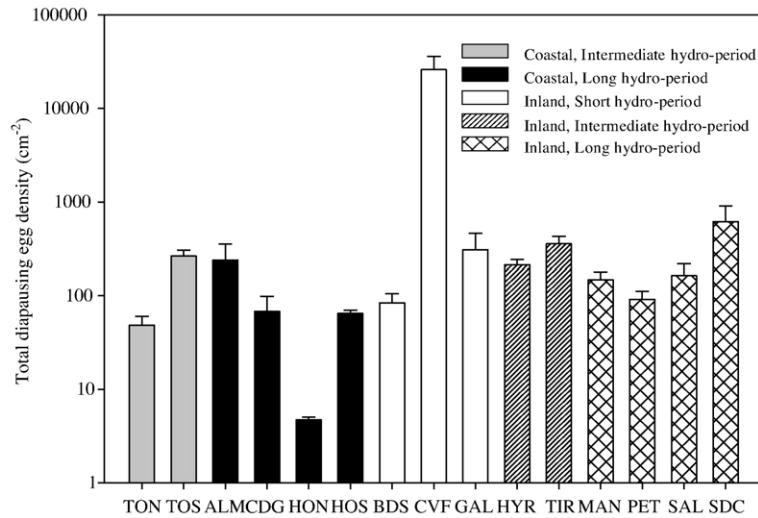[c] Model: Binomial, link function: logit, % Deviance: 81.9.

Fig. 4. Total diapausing egg density (cm^{-2}) for the uppermost 10 cm of sediment depth of the studied ponds. Location (Coastal vs. Inland) and hydro-period duration (Short, intermediate and long) of the studied ponds is indicated by different shading. Values are means of three sampling points per pond+1 S.E.

ponds; 8793 ± 5176 eggs cm^{-2}) had greater diapausing egg banks than ponds with hydro-periods of medium (i.e., seasonal; 220 ± 38 eggs cm^{-2}) and long (i.e., semi-permanent and permanent; 174 ± 50 eggs cm^{-2}) duration. This seems to be due to the effect of Camino de

Villafranca, as suggested by the fact that the range for long hydro-period, inland ponds encompasses the ranges of the rest of inland ponds.

Density values of 'hatched' and 'healthy-looking' eggs represented a small fraction of the egg bank.
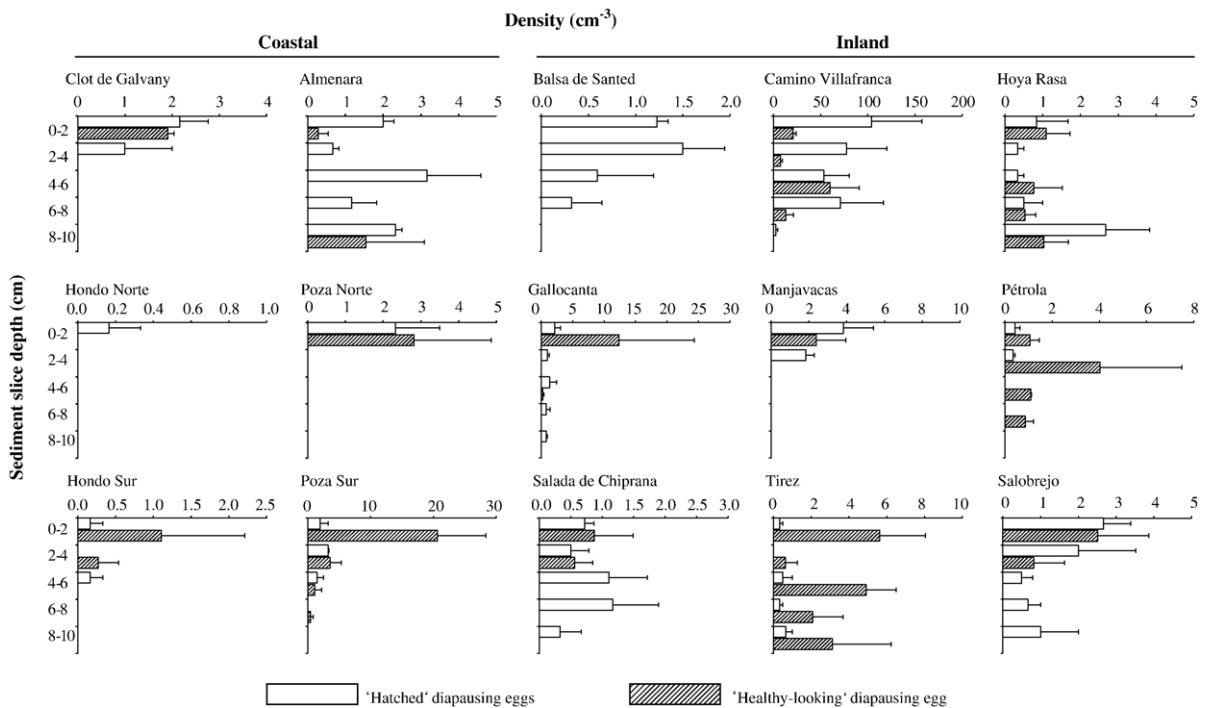
Fig. 5. Density of 'hatched' and 'healthy-looking' diapausing eggs of the *B. plicatilis* species complex at different depths in the sediments of the studied ponds. Data for each sediment layer are means for three sampling stations+1 S.E. Note the different scales on the *x*-axes.

Table 3
Sedimentation rates (cm year^{-1}) based on ^{210}Pb and ^{137}Cs dating methods

Pond	Sedimentation rate (cm year^{-1})			Maximum egg age interval (years)	Median age (years)
	^{210}Pb	^{137}Cs	Assumed		
Clot de Galvany	0.04–0.08	∼ 0.07	0.06	0–33.3	16.7
Camino de Villafranca	0.09–0.13	0.05–0.10	0.10	60–80	30
Salobrejo	0.15–0.17	0.10–0.15	0.15	13.3–26.7	6.7
Gallocanta	0.27–0.35	–	0.31	12.9–19.4	3.2

Maximum egg age interval and median age of 'healthy-looking' eggs are also indicated. In order to calculate egg age, we assumed either the more accurate value or the central value of the overlapping range between the two dating methods.

Percentage of 'deteriorated' eggs ranged from 74.6% in Poza Sur to 98.5% in Salada de Chiprana, the average being 89.1±1.7%. 'Healthy-looking' diapausing eggs represented a 5.9±1.4% of the egg bank. The maximum recorded was 19.1% in Poza Sur. In the ponds where 'healthy-looking' diapausing eggs were detected, densities for the integrated upper 10 cm of sediment ranged from 2.74 eggs cm^{-2} (Salada de Chiprana) to 200 eggs cm^{-2} (Camino de Villafranca). No 'healthy-looking' diapausing egg was detected at any sampling station in either Hondo Norte and Balsa de Santed. 'Hatched' diapausing eggs in our samples was 5.0±0.8% of total eggs. 'Hatched' eggs were found in the sediments of all the ponds studied.

'Healthy-looking' diapausing eggs were present even at the deepest sediment layer studied (8–10 cm) in some of the ponds sampled (Fig. 5). However, the majority of these eggs (62.4±9.6%) was typically restricted to the upper (0–2 cm) layer of sediment, and so assumed to be young. This was confirmed for some of our studied ponds after dating analysis. Six sediment cores were scheduled for dating (see Methods). In three cases (Salada de Chiprana, Poza Sur and Hondo Sur), the tested cores did not match the expected ideal profiles for both ^{210}Pb and ^{137}Cs patterns, so they did not provide dating information. Radio-nuclide profiles for Clot de Galvany, Camino de Villafranca and Salobrejo fitted the standard expectations. ^{210}Pb and ^{137}Cs methods gave slightly different sedimentation rate estimations, so the more accurate value (lower standard error) or the mid-point of the overlap between the ranges yielded by the two methods, was assumed to calculate egg age. Data for Gallocanta is available from the literature. Table 3 shows sedimentation rate estimations and the maximum egg age interval where 'healthy-looking' diapausing eggs were found. The median age of these eggs indicates that most of the 'healthy-looking' eggs were relatively young when compared to maximum age values. When three ponds in which egg age was

estimated are compared, they show large differences in the age of the fraction of 'healthy-looking' eggs. While the maximum estimated egg age in Gallocanta is 13–20 years, it reached 60–80 years in Camino de Villafranca. Fig. 6 shows the average distribution of the proportion of 'healthy-looking' eggs with depth (age). We found a decreasing pattern which was interpreted as an ageing effect of these eggs. No clear trend with depth was observed for 'hatched' eggs, but they were typically less frequent as sediment depth increased. This is probably due to the degradation of the egg shell remains over time.

GLMs on the ratios 'healthy-looking' to 'hatched' eggs (He/Ha) and 'healthy-looking' to 'deteriorated' eggs (He/D) in the upper 2 cm of sediment showed that ponds differed in hatching and deterioration patterns. GLMs detected significant effects due to location and hydro-period pattern (Table 2). However, these effects seem largely due to specific ponds. For instance, coastal ponds showed on average higher values for He/Ha than inland ponds (coastal, 7.38± 3.89 S.E.; inland, 4.44±1.96 S.E.), but the range in the former includes the range in the latter, and each of the two ponds with highest He/Ha belong to a
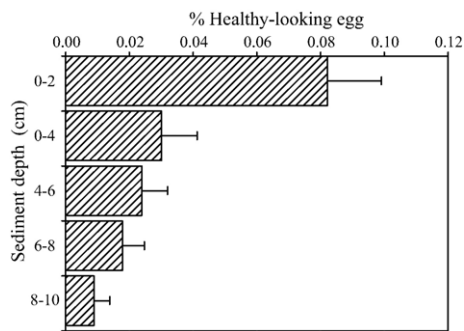

Fig. 6. Sediment depth distribution of the proportion of 'healthy-looking' diapausing eggs. Data for each sediment layer are means for all ponds+1 S.E.
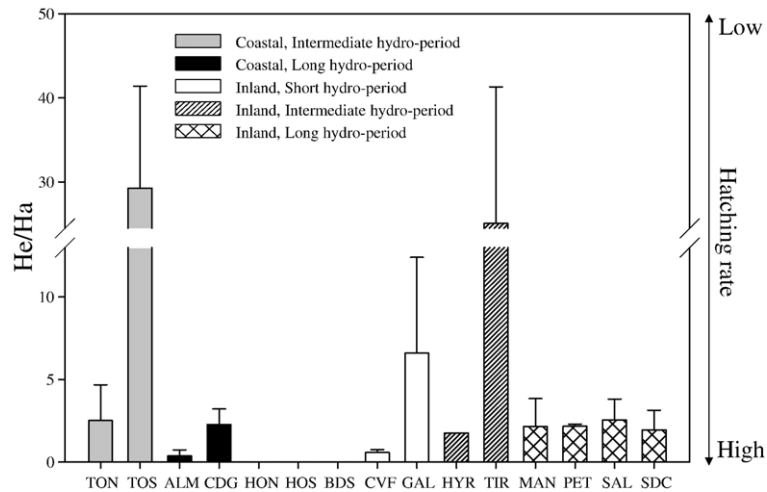
Fig. 7. Differences in the ratio between 'healthy-looking' and 'hatched' diapausing eggs (He/Ha) for the uppermost 2 cm of sediment depth of the studied ponds. Notice that this ratio is inversely related to the hatching rate of eggs in the sediment (see text). Location (Coastal vs. Inland) and hydro-period duration (Short, intermediate and long) of the studied ponds is indicated by different shading. Values are means of three sampling points per pond+1 S.E.

different area. Intermediate hydro-periods, when averaged, exhibited the highest He/Ha values in both coastal and inland ponds (16.36±6.32 S.E.), but it seems to be due to two particular ponds: Poza Sur and Tírez (Fig. 7). He/D was higher in coastal (0.17± 0.07 S.E.) than in inland ponds (0.08±0.02 S.E.), but again the range of the former embraces the range in the latter (Fig. 8), and a single pond (Poza Sur) weights largely in the higher average ratio He/D for intermediate hydro-periods (0.24±0.09 S.E.).

3.2. Relating diapausing egg bank properties and habitat conditions

A principal components analysis performed to explore the relationships between egg bank properties and habitat conditions gave two major factors explaining 63.6% of total variance (Fig. 9). The first axis (43.3%) correlated positively with the three egg abundance measurements and with salinity, area and short hydroperiods. The second axis (20.3%) was
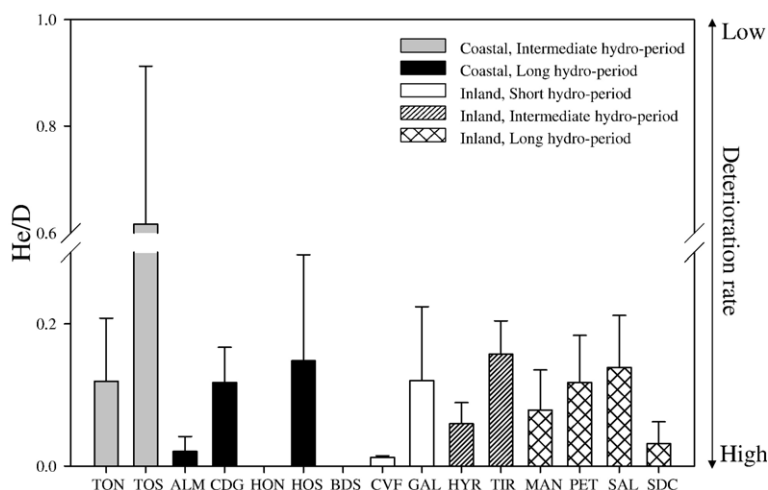

Fig. 8. Differences in the ratio between 'healthy-looking' and 'deteriorated' diapausing eggs (He/D) for the uppermost 2 cm of sediment depth of the studied ponds. Notice that this ratio is inversely related to the deterioration rate of eggs in the sediment (see text). Location (Coastal vs. Inland) and hydro-period duration (Short, intermediate and long) of the studied ponds is indicated by different shading. Values are means of three sampling points per pond+1 S.E.
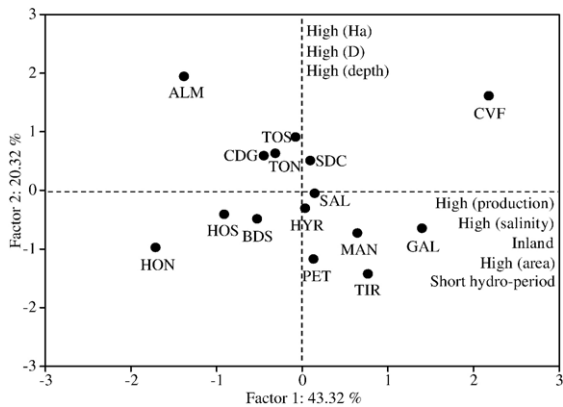
Fig. 9. Location of the studied ponds in the space defined by the first two factors in the principal components analysis. Correlation coefficients on first axis for: log(D)=0.821, log(He)=0.660, log(Ha)=0.658, log(salinity)=0.762, log(area)=0.616, location=0.684, hydro-period=−0.568. Correlation coefficients on second axis for: log(D)=0.508, log(He)=0.271, log (Ha)=0.639, and log(depth)=0.681.

positively related to 'hatched' and 'deteriorated' egg abundances, the correlation with 'healthy-looking' egg abundance being weak. Pond depth was the only habitat feature correlated ($r > 0.5$) with this axis.

4. Discussion

Compared with other zooplankters, rotifers have shorter generation times and faster growth, their populations are almost always temporary, and they are regarded as relatively vulnerable to biotic interactions (i.e., predation and competition). Since these features could have implications for the natural selection and constraints acting on dormancy, a question arises as to whether patterns in diapausing egg banks observed in other zooplankters can be generalized to monogonont rotifers. The present study gives information on this issue, by providing for the first time extensive quantitative information for the demography of rotifer diapausing egg banks.

Literature on zooplankton diapause egg banks has focused on describing and analysing 'healthy-looking' diapausing eggs (Marcus, 1990; Hairston et al., 1995; Cáceres, 1998), which are assumed to represent the fraction of eggs that remain viable in the sediments. Our results indicate the presence of a substantially large diapausing egg bank in almost all of the ponds studied. In our study, excluding two cases where 'healthy-looking' eggs were not detected, the density of these eggs ranged 2–115 cm^{-2}, which is in the same range as for other zooplankters (1–1000 cm^{-2}, see Hairston, 1996).

Similarly to other zooplankters, rotifer 'healthy-looking' eggs showed a variety of patterns with sediment depth. Their vertical distribution in the studied ponds is complex, not always decreasing, pointing out that the factors determining diapausing egg distribution vary even at a regional and local scale, for a low-level rotifer taxon. For instance, some vertical patterns with conspicuous deep peaks of egg abundance suggest large among-year variation in diapausing egg production. A large among-year variation is not surprising in shallow ponds in a Mediterranean region, since the populations inhabiting them can be greatly affected by climate fluctuations and local weather effects. This among-year variation is also suggested by deep peaks of total diapausing eggs, a variable more directly related to diapause egg production. Non-decreasing abundances of these types of eggs are unlikely due to sediment mixing. Sediment mixing would have result in a rather homogeneous distribution of eggs with depth, and unclear depth-structuring of egg conservation stages, two features that are not in agreement with our observations. Despite the cases of non-decreasing 'healthy-looking' diapausing egg abundance, these eggs were remarkably less frequent in deeper sediments, and a strictly decreasing pattern was found in some ponds. This suggests an ageing effect better shown by the decreasing pattern of the percentage of 'healthy-looking' eggs; i.e., when the effect of variation in total number of eggs is cancelled. This ageing effect is also supported by the lack of a declining pattern in the case of 'hatched' eggs.

'Deteriorated' eggs were found even at top sediment layers; consequently we stress that diapausing egg deterioration in the studied ponds is fast. The fact that most 'healthy-looking' eggs were collected in the upper sediment layers, besides our estimated sedimentation rates, suggests that diapausing egg banks in our ponds are young, the median age ranging from 3 to 30 years in the dated sediments. Maximum egg age was 60–80 years, which is close to other previous reports in rotifers (65 years; Kotani et al., 2001), but much lower than the highest longevities reported in cladocerans (125 years; Cáceres, 1998) or copepods (up to 300 years; Hairston et al., 1995). Nevertheless, the egg bank age in our study might be dependent on habitat features (i.e., ponds, most of them very shallow and non-permanent), instead being a differential characteristic of rotifers.

A striking result of our study is that 'healthy-looking' diapause eggs and 'hatched' eggs are both small fractions of total diapausing eggs. This fact could be observed because, for the first time in a quantitative

report for zooplankton, different types of diapausing eggs, instead of only 'healthy-looking' ones, were counted. Our results show that the most frequent fate of a rotifer diapausing egg seems to be deterioration (approx. 90%), which supports the view that sediment is not so safe as previously thought. Since diapausing egg deterioration in the sediment may be different depending on the pond, these differences should be taken into account in order to hypothesize optimal hatching patterns (García-Roger et al., 2006), as discussed below.

Data on total egg bank (i.e., 'healthy-looking', 'deteriorated' and 'hatched' eggs) should reflect the performance of the active population in a habitat more directly than the abundance of any particular egg type. It should be noted that rotifer populations usually inhabit temporary habitats and their survival depends on the production of diapausing eggs. Therefore, although a trade-off between current growth and the production of diapausing stages exists during the active, zooplanktonic period, it is expected that diapausing egg production will tend to be maximized yearly, because it is the between-year fitness measurement (King and Serra, 1998). As it has been advanced, total egg bank is an estimation of diapausing egg net production, from which losses due to early predation (e.g., in the water column) or exportation (i.e., dispersion) are subtracted. Notice that the latter could be particularly important in ponds that dry during long periods (i.e., highly ephemeral ponds) since eggs may be blown away by wind (Langley et al., 2001; Cáceres and Soluk, 2002), and might be affecting our data. Nevertheless, the putative egg loss caused by these factors should be considered an adversity–i.e., lack of performance–for population persistence.

Our data show the existence of large differences in total egg bank size among ponds, and thus in population performance. Inland ponds produced on average more diapausing eggs than coastal ponds. This is probably due to the fact that inland ponds are endorheic, highly saline–a condition favouring B. plicatilis complex–and rich in nutrients. It is known that the species of the B. plicatilis complex are commonly dominant in the rotifer community of the inland ponds studied here, whereas the latter is not the case for most of the also very productive coastal ponds (Lapesa, 2004). PCA suggested salinity, an environmental factor with higher values in inland ponds, as a predictor of the general differences in habitat quality when inland and coastal habitats are compared. Both the results from GLM and PCA suggest that diapausing egg production (He + Ha + D) is related to short hydro-periods, while PCA detected an association to pond area, an indicator of

stability. The former association might be largely due to a single case, the large bank found in Camino de Villafranca, a short hydro-period pond. However, a more general effect cannot be ruled out, since the lowest bank was found in a long hydro-period pond (Hondo Norte).

Expectations are that natural selection maximizes diapausing egg production and minimizes egg deterioration. By contrast, the hatching fraction should be under optimizing selection, and responding to a balance between risks of hatching (i.e., hatching under unsuitable conditions in the water column) and fitness loss caused by remaining in the sediment (i.e., deterioration). All other things being equal, we expect that the higher the risk in the water column, then the lower the hatching fraction (Cohen, 1966). However, all other things might not be equal. Our data provide evidence that deterioration rates of the eggs in the sediment are high and different in different habitats ('deteriorated' egg proportion ranged from 74.6% to 98.5%). Diapausing eggs in the sediments of shallow ponds, if compared to deep lakes, might be exposed to extreme, environmental conditions (e.g., desiccation, compacting pressure while buried in the sediment, high exposure to radiation, damages produced by salt crystals when the pond dries out, predators, etc.) which may accelerate deterioration processes. Another observed relationship, although difficult to interpret, is that deterioration is lower in ponds with intermediate hydro-periods. As a result of our findings, we conclude that among-habitat and among-species variation in deterioration rates should be taken into account in order to interpret diapausing egg bank composition and predict optimal hatching rates.

The ratio 'healthy-looking' to 'hatched' (He/Ha) eggs is inversely related to hatching rate, and, under some sound simplifying assumptions, is unaffected by production and deterioration rates (see García-Roger et al., 2006). A clear effect of area and hydro-period on hatching rates (as estimated by He/Ha) has not been found, the significance detected by GLM being likely due to the effect of specific ponds. However, PCA gives some insights into the factors shaping hatching rates, since this exploratory analysis conveniently separates in different axes measurements that are indicative of net egg production (first axis) from measurements that can be interpreted as related to other processes. The second axis is positively related to 'hatched' and 'deteriorated' egg abundances, but weakly related to 'healthy-looking' egg abundance. Therefore, this axis is associated with sediments where an excess of 'deteriorated' and 'hatched' egg exists, in relation to what would be

expected from the amount of 'healthy looking' eggs. Such excesses are, respectively, indicative of high rates of deterioration and hatching. Interestingly, these results provide the first empirical evidence that both rates are associated. As stated above, high hatching rates are advantageous when the right timing for hatching can be accurately predicted, but also when sediment is risky. In case of risky sediments, a higher hatching rate might be selected even if the water column is highly unpredictable.

Acknowledgements

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