Freshwater Biology (2006) **51,** 1351–1358 doi:10.1111/j.1365-2427.2006.01583.x

Hatching and viability of rotifer diapausing eggs collected from pond sediments

EDUARDO M. GARCÍA-ROGER, MARÍA JOSÉ CARMONA AND MANUEL SERRA
Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, València, Spain

SUMMARY

1. Planktonic rotifers inhabiting variable environments produce diapausing eggs that accumulate in the sediment of lakes and ponds, forming egg banks that may withstand adverse periods. A common assumption in zooplankton diapausing egg bank studies is to count as viable all eggs in the sediment that look healthy. This assumption should be challenged by asking how effectively 'healthy-looking' eggs represent viable eggs.
2. In this study, viability of more than 1100 'healthy-looking' diapausing eggs belonging to the *Brachionus plicatilis* species complex was assessed in a laboratory hatching experiment. Eggs were collected at different depths from sediment cores obtained from 15 ponds located in coastal and inland areas of Eastern Spain.
3. Only approximately one half of the 'healthy-looking' diapausing eggs hatched after incubation in experimental conditions. Almost all the hatchlings (99.4%) survived to maturity. The proportion of 'healthy-looking' diapausing eggs that hatched varied among areas and among ponds within area, and substantially declined with sediment depth. Most of the hatchlings (88%) were obtained from the uppermost 2 cm of sediment. 'Healthy-looking' eggs from upper sediment layers hatched after significantly shorter incubation times than eggs recovered from deeper layers.
4. Both decreased hatching success and increased incubation time for hatching with sediment depth suggest that older 'healthy-looking' eggs are less responsive to hatching stimuli and could become unviable. However, the strong correlation found between the number of 'healthy-looking' eggs and the number of hatchlings indicates that the abundance of 'healthy-looking' eggs is a good index of egg bank viability.

Keywords: diapausing eggs, hatching, rotifers, sediment, viability

Introduction

Planktonic rotifers inhabiting variable environments are exposed to abiotic and biotic conditions threatening their survival and reproduction. In almost all species, production of diapausing eggs is coupled to the sexual phase of their cyclical parthenogenetic life cycles (e.g. Wallace & Snell, 1991). Diapausing eggs resist adverse conditions and when conditions in the water column are suitable, their hatching allows population growth to resume. A fraction of the diapausing eggs do not hatch when the conditions are favourable, which results in a pool of diapausing eggs, the so-called 'egg bank' (De Stasio, 1989; Marcus *et al*., 1994; Hairston, 1996). The egg bank, analogous to plant seed banks, has important implications for ecology, genetics and evolution of rotifer populations (Templeton & Levin, 1979; Hairston, 1998; Ortells *et al*., 2000; Gyllström & Hansson, 2004).

The rotifer *Brachionus plicatilis* (Müller 1786) is considered as a worldwide inhabitant of brackish or saline lakes and ponds (Walker, 1981; Hammer, 1986). This taxon is a complex of a still undetermined number

Correspondence: Eduardo M. García-Roger, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, AO 22085, València 46071, Spain.
E-mail: eduardo.garcia@uv.es

of closely related species (Gómez, Temprano & Serra, 1995; Ortells *et al.*, 2000; Gómez *et al.*, 2002; Suatoni, 2003). In Spain, the species of the *B. plicatilis* complex form diapausing egg banks in a wide range of ponds and lakes (Ortells *et al.*, 2000; García-Roger, Carmona & Serra, 2005). Estimates of viability of diapausing eggs in these sediment banks are needed in order to assess the influence of recruitment from the bank on the active populations. Typically, egg viability is established on the basis of their aspect. Morphologically intact, apparently well-preserved diapausing eggs (i.e. 'healthy-looking' eggs, see criteria in García-Roger *et al.*, 2005) are assumed to represent the fraction of eggs that remain viable in the sediments. This is a common approach in the study of zooplankton banks (Marcus, 1990; Hairston *et al.*, 1995; Cáceres, 1998; Keller & Spaak, 2004). However, the estimation of hatching success of these eggs is an open question, which is relevant in estimating the effective size of the egg bank and its potential for recruitment to the active population.

The aim of the present study is to experimentally address the hatching success of apparently viable ('healthy-looking') diapausing eggs of the *B. plicatilis* species complex isolated from the sediment banks in a wide range of ponds and lakes located in Eastern Spain. In our study we included a variety of habitats because we wanted to explore if hatching success is correlated to habitat features.

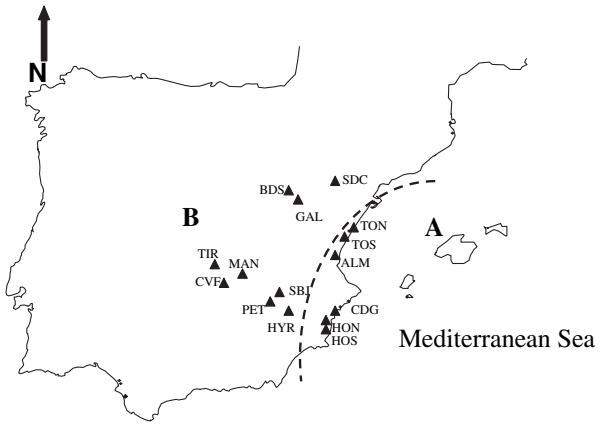

Fig. 1 Map of Spain showing the location of the studied ponds. (a) Coastal: ALM, laguna de Almenara (short. Almenara); CDG, Clot de Galvany; HON, Charca Norte, 'El Hondo de Elche' Natural Park (short. Hondo Norte); HOS, Charca Sur, 'El Hondo de Elche' Natural Park (short. Hondo Sur); TON, Poza Norte, Cabanes-Torreblanca Marsh; TOS, Poza Sur, Cabanes-Torreblanca Marsh. (b) Inland: BDS, Balsa de Santed; CVF, Laguna del Camino de Villafranca (short. Camino Villafranca); GAL, Laguna de Gallocanta (short. Gallocanta); HYR, Hoya Rasa; MAN, Laguna de Manjavacas (short. Manjavacas); PET, Laguna de Pétrola (short. Pétrola); SDC, Salada de Chiprana; SBJ, Laguna del Salobrejo (short. Salobrejo); TIR, Laguna de Tírez (short. Tírez). Dashed line in the map separates both areas.

Methods

Study system

Diapausing eggs of the *B. plicatilis* species complex were collected from the sediments of 15 ponds and lakes Eastern Spain (Fig. 1). Ponds were classified as coastal (six ponds: Almenara, Clot de Galvany, Hondo Norte, Hondo Sur, Poza Norte, Poza Sur) or inland (nine ponds: Balsa de Santed, Camino de Villafranca, Gallocanta, Hoya Rasa, Manjavacas, Pétrola, Salada de Chiprana, Salobrejo, Tírez). In these ponds, diapausing egg banks of the *B. plicatilis* species complex had been previously detected (Ortells *et al.*, 2000; García-Roger *et al.*, 2005). The sampled habitats are representative of the great diversity of salt and brackish ponds and lakes found in the Iberian Peninsula. The habitats varied in size (from 70 m^2, Poza Norte, to 13.3 km^2, Gallocanta), depth (almost all ponds being very shallow, <1 m in average depth, with the exception of Almenara, Salobrejo and Salada de Chiprana, which have a maximum depth higher than 3 m), salinity (from oligohaline, 2 g L^{-1} in Almenara, to hypersaline, more than 150 g L^{-1} in Tírez) and water permanence conditions (ephemeral, seasonal, semipermanent and permanent ponds), thus representing a wide ecogeographic range where *B. plicatilis* species occur.

Sediment sampling

Sediment samples were collected during summer 2001 from three randomly selected sampling points in each pond or lake by means of a piston core sampler (57 mm internal diameter, 60 cm length; Ejkelkamp Agrisearch Equipment, Giesbeek, The Netherlands). Each core was sliced in 2-cm sections down to a maximum depth of 10 cm. Slices were packed in 100-mL plastic containers, labelled and stored at 4 °C in the dark until processed. These conditions went on for 2 months, which is the time span needed to complete the mandatory refractory period for hatching

induction in recently produced diapausing eggs of the *B. plicatilis* (Hagiwara & Hino, 1989).

Isolation of diapausing eggs

Diapausing eggs were isolated using the sucrose flotation technique developed by Onbé (1978) and modified by Gómez & Carvalho (2000). This technique facilitates obtaining large quantities of diapausing eggs in order to calculate reliable hatching success rates and no bias related to the extraction of eggs in different conservation status has been observed (García-Roger *et al*., 2005). For egg isolation, a sediment subsample of 10 g (wet weight) was taken from the central cylinder of each 2-cm sediment slice. Each subsample was resuspended in 1.75 mol L^{-1} sucrose solution in 45 mL centrifuge tubes and centrifuged at 12.6 ***g*** for 5 min. The supernatant was washed thoroughly through a 200-μm and a 30-μm Nytal mesh. The former mesh retained large material (e.g. small remnants of plants and animals), while diapausing eggs of the *B. plicatilis* species complex (length: 113–137 μm; width: 75–100 μm; Ciros-Pérez, Gómez & Serra, 2001) were retained by the 30-μm mesh. The 30-μm retained material was transferred to a plankton counting chamber for diapausing egg identification and isolation. Only well-preserved, 'healthy-looking' diapausing eggs (orange-brown eggs exhibiting envelopes and embryo content both intact; for a detailed description see García-Roger *et al*., 2005) were selected for hatching experiments.

Hatching experiments

In order to induce hatching, 'healthy-looking' diapausing eggs were individually incubated at 25 °C under constant illumination (150–170 μmol quanta m^{-2} s^{-1}) in multi-well plates containing 200 μL of synthetic seawater (Instant Ocean®; Aquarium Systems, Inc., Mentor, OH, U.S.A.) at 6 g L^{-1}. This salinity was selected as it has been used successfully for hatching induction in diapausing eggs belonging to the *B. plicatilis* species complex in previous studies (Gómez *et al*., 2002; Ortells, 2002). Wells were checked for hatchlings at 12-h intervals during 14 days. As the hatching of diapausing eggs of the *B. plicatilis* species complex has also been successfully induced at lower salinity values (Ortells *et al*., 2000), unhatched eggs, which still looked healthy, were individually transferred to new plates containing 2 g L^{-1} salinity fresh medium, the rest of the conditions remaining the same and were monitored for seven additional days. In the hatching experiments, the medium was renewed every other day during incubation in order to prevent fungal infection.

Statistical analyses

The hatching success of 'healthy-looking' diapausing eggs was analysed using a generalised linear model (GLM) with binomial errors and logit as link function (Nelder & Wedderburn, 1972). This model assessed for variation because of location (coastal versus inland), pond within location and sediment depth. The goodness of fit of the model was assessed by the percentage of deviance explained by the model with respect to the deviance of the null model (i.e. model including as many parameters as observations).

Data on the time of hatching of diapausing eggs was not normally distributed and nonparametric Kruskal–Wallis rank sum tests were performed in order to evaluate the differences in hatching time among locations, ponds and sediment depths. When comparing differences in the time of hatching among ponds, only those with sample sizes higher than 20 hatchlings were used in the analysis. In the case of the sediment depth analysis, data were grouped into three depth ranges to increase sample sizes (0–2, 2–4 and 4–10 cm depth).

All statistical analyses were performed using R 2.1.0 statistical software (Ihaka & Gentleman, 1996).

Results

Hatching success

A total amount of 1119 'healthy-looking' diapausing eggs were isolated from the sediment samples of 13 of the 15 ponds and lakes studied (no 'healthy-looking' diapausing eggs were found in Hondo Norte and Balsa de Santed, so these ponds were excluded from our analysis). From these eggs, 47.5% hatched after incubation in the experimental conditions (i.e. a total amount of 532 hatchlings). Hatchling survival was high; 99.4% of hatchlings survived more than 48 h, which is sufficient time for *B. plicatilis* to become reproductively mature (Temprano *et al*., 1994).

Table 1 Statistical results of the generalised linear model fitted to hatching success data

Source	d.f.	Deviance	*P*-value
Location	1	55.86	<0.001
Depth	4	126.69	<0.001
Pond (location)	11	229.32	<0.001
Location × depth	4	13.26	0.01
Pond (location) × depth	20	93.24	<0.001

Model: binomial; link function: logit; % deviance: 74.

Hatching success varied considerably among ponds. Average hatching success for the integrated top 10 cm sediment in the 13 ponds was 38.8% (±7.3 SE), the maximum value of hatching success being 72.7% (Poza Norte). No hatchlings were found in Gallocanta and Hoya Rasa. A GLM comparing hatching success for the upper 10 cm of sediment (Table 1) showed significant differences because of pond location (coastal ponds showed higher hatching rates than those inland), as well as differences among ponds within a given location. Hatching success in coastal ponds was, on average, 49.9% (±12.8 SE), whereas it was 31.8% (±7.8 SE) in inland ponds. Differences in hatching success between diapausing eggs isolated from inland and coastal ponds were not the result of particular ponds or lakes accounting for extremely high or low hatching success values. As the studied ponds differ in salinity, we explored whether hatching success of diapausing eggs was related to pond salinity. Correlation was not significant ($r = 0.112$, $n = 13$, $P = 0.715$).

The GLM also revealed the significant effect of depth (Table 1). Fig. 2 shows that hatching success of 'healthy-looking' eggs declined with the sediment depth from which they were isolated. It varied from 57.8% in the upper 0–2 cm sediment layer to 6% at the deepest 8–10 cm sediment layer studied. Because of this pattern and to the fact that 'healthy-looking' eggs tended to be more abundant in the upper sediment layer, most of the hatchlings (88.0% ± 6.4% SE, after averaging for the 11 ponds where hatchlings were observed) were obtained from the uppermost 2 cm of sediment.

Fig. 3 shows the relationship between the number of 'healthy-looking' diapausing eggs of each pond exposed to hatching conditions and the number of hatchlings obtained from the uppermost 2 cm of sediment. Although not all the 'healthy-looking' diapausing eggs were able to hatch, we found a strong correlation between both variables ($r = 0.940$, $n = 13$). It is worth noting that although the number of 'healthy-looking' diapausing eggs and the number of hatchlings are not independent variables, the correlation between the number of hatchlings and the number of unhatched diapausing eggs was not significant ($r = 0.229$, $n = 13$, $P = 0.453$), which

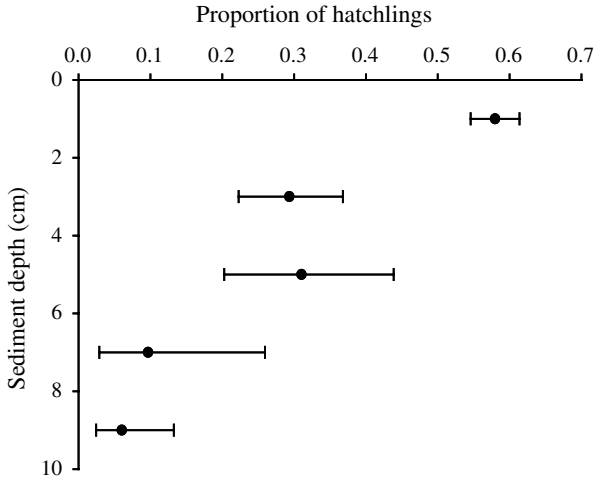

Fig. 2 Relationship between the proportion of 'healthy-looking' diapausing eggs that hatched and the sediment depth. Values for each depth were obtained from the grouped data of the whole set of ponds studied. Bars are 95% shortest unbiased confidence limits for proportions (Rohlf & Sokal, 1995).

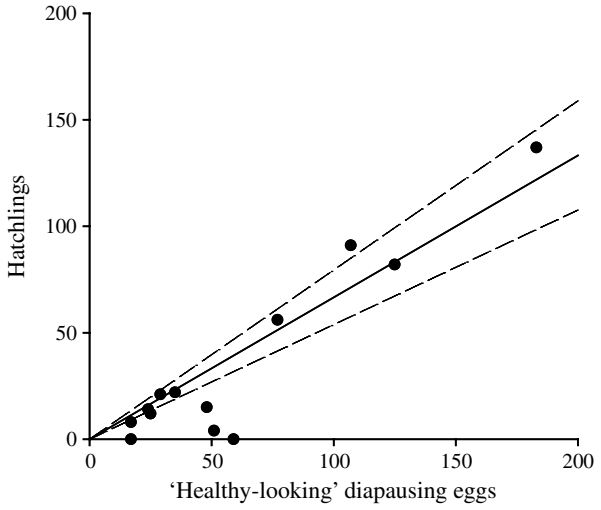

Fig. 3 Relationship between the number of 'healthy-looking' diapausing eggs and the number of hatchlings obtained for each pond studied. The solid line is the regression for all ponds (slope = 0.666, $r = 0.940$, $n = 13$). Dashed lines indicate 95% confidence limits.

indicates that the hatching fraction is not dependent on the number of isolated 'healthy-looking' diapausing eggs.

Time of hatching

We did not find significant differences in the distribution of hatching time of diapausing eggs isolated from coastal or inland pond sediments (Kruskal–Wallis $\chi^2 = 0.178$, d.f. $= 1$, $P = 0.674$). By contrast, we found significant differences when ponds were individually compared (Kruskal–Wallis $\chi^2 = 44.68$, d.f. $= 7$, $P \ll 0.001$). Note that only ponds with sample sizes higher than 20 hatchlings were used in this analysis (eight ponds).

Fig. 4 shows the hatching dynamics of diapausing eggs isolated from the different sediment depth layers. All but three hatchlings occurred within the first 200 h of incubation. These three hatchlings (observed at 216, 228 and 360 h) were from eggs in the 0–2 cm sediment layer, in which the remaining hatchlings occurred within 180 h of incubation. After grouping the data in three depth intervals (0–2 cm, 2–4 cm and 4–10 cm) to increase sample size, a Kruskal–Wallis rank sum test comparing the hatching times for the sediment depth layers revealed the existence of significant differences ($\chi^2 = 22.29$, d.f. $= 2$, $P \ll 0.001$). In the three groups, the median hatching time was 36 h. The average hatching time of diapausing eggs decreased with sediment depth, from 39.47 ± 1.16 h in the 0–2 cm sediment layer, to 49.85 ± 4.82 h for the 2–4 cm sediment depth layer and 53.28 ± 5.07 h in the 4–10 cm sediment depth layer.

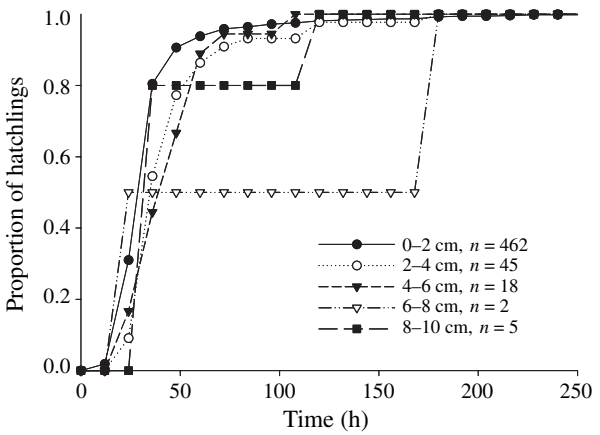

Fig. 4 Cumulative proportion of hatchlings with time for the different sediment depths studied. Values for each sediment depth were obtained from the grouped data of the whole set of ponds studied.

Discussion

A common practice in zooplankton diapausing egg bank studies is to assess as viable all the eggs in the sediment that look healthy (Hairston *et al.*, 1995; Weider *et al.*, 1997; Cáceres, 1998; Keller & Spaak, 2004). Although an egg is only proven to be viable if it hatches into a live individual, the use of egg appearance as a viability criterion facilitates egg bank surveys. Laboratory hatching experiments are time–consuming and the lack of knowledge about hatching cues in some taxa makes setting hatching conditions difficult (Schwartz & Hebert, 1987; Tsitsilas & Barry, 2002). Moreover, the existence of intraspecific variation in the hatching response (De Meester & De Jager, 1993; De Stasio, 2004) has been considered to detract from the reliability of hatching experiments (Cáceres, 1998). However, when assessing egg viability by means of egg appearance, an important question is how well 'healthy-looking' eggs represent viable eggs. Our results show that only half of the 'healthy-looking' eggs isolated from *B. plicatilis* complex sediment egg banks successfully hatched in the laboratory. Diapausing egg hatching success will depend on (i) the completion of the mandatory period of diapause, (ii) the suitability of the experimental conditions inducing egg hatching and (iii) the viability of the diapausing eggs. Our experiment was designated to maximise diapausing eggs hatching. First, care was taken to ensure that all the eggs had completed the mandatory period of diapause, and second, the set of conditions that we applied had been successfully used in previous studies to induce hatching in diapausing eggs of *B. plicatilis*. These conditions were: (i) the extraction of the diapausing eggs from the sediments, which is thought to enhance hatching as eggs would be subjected to better conditions than those still buried (Vandekerkhove *et al.*, 2004), (ii) a putatively optimal temperature of 25 °C (Hagiwara, Hino & Hirano, 1985) and (iii) illumination, which has been recognised in laboratory experiments to be an obligatory cue for the break of diapause in *B. plicatilis* and some other co-generic rotifer species (Minkoff, Lubzens & Kahan, 1983; Hagiwara *et al.*, 1985; Hagiwara & Hino, 1989; Hagiwara *et al.*, 1995), although

exceptions in the genus *Brachionus* have been also reported (Pourriot & Snell, 1983; Bailey *et al.*, 2003). Our experimental design also included the sequential incubation of diapausing eggs at 6 and 2 g L^{-1} salinity, both of which have been proved to be adequate for the hatching of diapausing eggs belonging to the *B. plicatilis* species complex (Gómez *et al.*, 2002; Ortells, 2002). Thus, a high hatching success was expected in our experiment. We cannot exclude that a fraction of viable eggs may not have been able to hatch under these conditions, but unsuitability of hatching induction conditions seems unlikely to explain the hatching failure of so many 'healthy-looking' eggs as we observed in our experiment (52.5%). The declining of hatching success with sediment depth found in our experiment suggests that egg age plays a role in explaining this result. Hatching success in the uppermost 2 cm of sediment was 60%, but declined to 6% at the deepest sediment layer studied, as was to be expected if eggs lose their ability to hatch with age, even if keeping a 'healthy' look. We hypothesise that, in spite of their still healthy appearance, older eggs are less responsive to hatching stimulus. A decreasing pattern in hatching success with sediment depth (age) has also been observed in cladocerans and copepods by several researchers (Carvalho & Wolf, 1989; Hairston *et al.*, 1995; Weider *et al.*, 1997; Cousyn & De Meester, 1998). Cousyn & De Meester (1998) have suggested that photosensitive compounds, known to be stored in cladocerans eggs (Davison, 1969; Van der Linden *et al.*, 1991), would deteriorate with age. Such deterioration would make the diapausing egg less responsive to a lighting cue. This explanation could hold for *Brachionus* rotifers eggs which it has been suggested to also store phothosensitive compounds (Gilchrist & Green, 1962; Hagiwara & Hino, 1990). Interestingly, we observed in our hatching experiment a positive relationship between the time span needed to hatch and the depth from which diapausing eggs were isolated, with an important fraction of eggs isolated from deep sediment layers showing a quite delayed hatching. A similar pattern has also been observed in diapausing eggs of the copepod *Diaptomus sanguineus* (Hairston *et al.*, 1995).

Results from the present study suggest that diapausing eggs could lose their viability with age and still have a healthy look. This might compromise the use of appearance as a viability criterion in egg bank studies as the number of 'healthy-looking' eggs would overestimate the number of viable diapausing eggs in the sediments. However, the strong positive correlation found between the number of 'healthy-looking' diapausing eggs and their hatching success indicates that the abundance of 'healthy-looking' diapausing eggs is a good index to assess the size of viable egg banks in the sediments of ponds and lakes.

According to our results hatching success of 'healthy-looking' diapausing eggs from coastal ponds is higher than that of eggs from inland ponds, which might imply that 'healthy-looking' diapausing eggs in inland ponds have a higher decrease in viability. Nevertheless, we also found low hatching rates in some coastal ponds, so we hypothesise that the rate to which 'healthy-looking' diapausing eggs deteriorate without showing appearance changes may be pond-dependent. It might be thought that conditions used in our hatching experiment biased hatching rates differentially in different ponds. Salinity, a factor affecting hatching (Minkoff *et al.*, 1983), used in our experiments is closer to natural salinity conditions in coastal ponds (16.2 ± 4.5 SE; E.M. García-Roger, M.J. Carmona & M. Serra, unpublished data), which exhibited higher hatching rates, than to salinity conditions in inland ponds (40.5 ± 7.1 SE). However, pond salinity was not correlated with hatching success. Differences in hatching success among locations could be because of differences in the egg quality when produced, different sedimentation rates and diapausing egg ages or to sediment conditions affecting diapausing egg preservation (e.g. low oxygen levels, increasing H_2S concentrations, desiccation, extreme temperatures, light exposure). Additional research is needed in order to identify which factors are really affecting the differences among ponds. Interestingly, we found differences in the time of hatching among ponds, which suggest that deterioration rates of diapausing eggs varies among ponds (i.e. sediment conditions may not be the same for egg preservation in the set of ponds studied) or that the age of diapausing eggs from different ponds is different because of the variation of sedimentation rates. As pointed out above, it is to be expected that older eggs would be less responsive to hatching stimuli so they would exhibit longer hatching times.

In conclusion, our results suggest that diapausing eggs may lose their viability while still looking healthy. This fact has implications on the common

practice of using the appearance as a viability criterion as the number of 'healthy-looking' eggs will overestimate the number of viable eggs in the sediments. However, the correlation found among the healthy look of the egg and its capability to hatch indicates that the number of 'healthy-looking' eggs is useful to characterise the size of the viable egg banks for comparative purposes.

Acknowledgments

We would like to thank two anonymous reviewers for helpful comments on the manuscript. This study was partially supported by a grant from the Spanish Ministerio de Ciencia y Tecnología BOS2000–1451, a grant from the Conselleria d'Empresa, Universitat y Ciència (Generalitat Valenciana) GRUPOS2004/20 and by a fellowship to E.M. García-Roger by the Ministerio de Educación, Cultura y Deporte AP2000–3550.

References

Bailey S.A., Duggan I.C., van Overdijk C.D.A., Jenkins P.T. & MacIsaac H.J. (2003) Viability of invertebrate diapausing eggs collected from residual ballast sediment. *Limnology and Oceanography*, **48**, 1701–1710.

Cáceres C.E. (1998) Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. *Ecology*, **79**, 1699–1710.

Carvalho G.R. & Wolf H.G. (1989) Resting eggs in *Daphnia*. I. Distribution, abundance and hatching of resting eggs collected from various depths in lake sediments. *Freshwater Biology*, **22**, 459–470.

Ciros-Pérez J., Gómez A. & Serra M. (2001) On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* complex from Spain, with the description of *B. ibericus* n. sp. *Journal of Plankton Research*, **23**, 1311–1328.

Cousyn C. & De Meester L. (1998) The vertical profile of resting egg banks in natural populations of the pond-dwelling cladoceran *Daphnia magna* Straus. Archiv für Hydrobiologie, Special Issues. *Advances in Limnology*, **52**, 127–139.

Davison J. (1969) Activation of the ephippial eggs of *Daphnia pulex*. *Journal of general Physiology*, **53**, 565–575.

De Meester L. & De Jager H. (1993) Hatching of *Daphnia* sexual eggs. I. Intraspecific differences in the hatching responses of *Daphnia magna* eggs. *Freshwater Biology*, **30**, 219–226.

De Stasio B.T. Jr (1989) The seed bank of a freshwater crustacean: copepodology for the plant ecologist. *Ecology*, **70**, 1377–1389.

De Stasio B.T. Jr (2004) Diapause in calanoid copepods: within-clutch hatching patterns. *Journal of limnology*, **63** (Suppl. 1), 26–31.

García-Roger E.M., Carmona M.J. & Serra M. (2005) Deterioration patterns in diapausing egg banks of *Brachionus* (Müller, 1786) rotifer species. *Journal of Experimental Marine Biology and Ecology*, **314**, 149–161.

Gilchrist B.M. & Green J. (1962) Carotenoid pigments in Rotifera. *Nature*, **195**, 905–907.

Gómez A. & Carvalho G.R. (2000) Sex, parthenogenesis and the genetic structure of rotifers: microsatellite analysis of contemporary and resting egg bank populations. *Molecular Ecology*, **9**, 203–214.

Gómez A., Temprano M. & Serra M. (1995) Ecological genetics of a cyclical parthenogen in temporary habitats. *Journal of Evolutionary Biology*, **6**, 601–622.

Gómez A., Serra M., Carvalho G.R. & Lunt D.H. (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution*, **56**, 1431–1444.

Gyllström M. & Hansson L.A. (2004) Dormancy in freshwater zooplankton: induction, termination and the importance of benthic-pelagic coupling. *Aquatic Sciences*, **66**, 274–295.

Hagiwara A. & Hino A. (1989) Effect of incubation and preservation on resting egg hatching and mixis in the derived clones of the rotifer *Brachionus plicatilis*. *Hydrobiologia*, **186/187**, 415–421.

Hagiwara A. & Hino A. (1990) Feeding history and hatching of resting eggs in the marine rotifer *Brachionus plicatilis*. *Nippon Suisan Gakkaishi*, **56**, 1965–1971.

Hagiwara A., Hino A. & Hirano R. (1985) Combined effects of environmental conditions on the hatching of fertilized eggs of the rotifer *Brachionus plicatilis* collected from an outdoor pond. *Bulletin of the Japanese Society of Scientific Fisheries*, **51**, 755–758.

Hagiwara A., Hoshi N., Kawahara F., Tominaga K. & Hirayama K. (1995) Resting eggs of the marine rotifer *Brachionus plicatilis* Müller: development, and effect of irradiation on hatching. *Hydrobiologia*, **313/314**, 223–229.

Hairston N.G. Jr (1996) Zooplankton egg banks as biotic reservoirs in changing environments. *Limnology and Oceanography*, **41**, 1087–1092.

Hairston N.G. Jr (1998) Time travelers: What's timely in diapause research? Archiv für Hydrobiologie, Special Issues. *Advances in Limnology*, **52**, 1–15.

Hairston N.G. Jr, Van Brunt R.A., Kearns C.M. & Engstrom D.R. (1995) Age and survivorship of

diapausing eggs in a sediment egg bank. *Ecology*, **76**, 1706–1711.

Hammer U.T. (1986) *Saline Lake Ecosystems of the World*, *Monographiae Biologicae 59*. Dr. W. Junk. Publishers, Dordrecht, The Netherlands.

Ihaka R. & Gentleman R. (1996) R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, **5**, 299–314.

Keller B. & Spaak P. (2004) Nonrandom sexual reproduction and diapausing egg production in a *Daphnia* hybrid species complex. *Limnology and Oceanography*, **49**, 1393–1400.

Marcus N.H. (1990) Calanoid copepod, cladoceran, and rotifer eggs in sea-bottom sediments of Northern California coastal waters: identification, occurrence, and hatching. *Marine Biology*, **105**, 413–418.

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. *Limnology and Oceanography*, **39**, 154–158.

Minkoff G., Lubzens E. & Kahan D. (1983) Environmental factors affecting hatching of rotifer (*Brachionus plicatilis*) resting eggs. *Hydrobiologia*, **104**, 61–69.

Nelder J.A. & Wedderburn R.W.M. (1972) Generalized linear models. *Journal of the Royal Statistical Society A*, **135**, 370–384.

Onbé T. (1978) Sugar flotation method for sorting the resting eggs of marine cladocerans and copepods from sea-bottom sediment. *Bulletin of the Japanese Society of Scientific Fisheries*, **44**, 1411.

Ortells R. (2002) Diversidad genética y ecológica en especies crípticas de rotíferos: patrones y procesos. Ph.D. Thesis, University of Valencia, Valencia, Spain.

Ortells R., Snell T.W., Gómez A. & Serra M. (2000) Patterns of genetic differentiation in resting egg banks of a rotifer species complex in Spain. *Archiv für Hydrobiologie*, **149**, 529–551.

Pourriot R. & Snell T.W. (1983) Resting eggs of rotifers. *Hydrobiologia*, **10**, 213–224.

Rohlf F.J. & Sokal R.R. (1995) *Statistical Tables*, 3rd edn. Freeman, San Francisco.

Schwartz S.S. & Hebert P.D.N. (1987) Methods for the activation of the resting eggs of *Daphnia*. *Freshwater Biology*, **17**, 373–379.

Suatoni L. (2003) Patterns of speciation in the rotifer species complex, *Brachionus plicatilis*. PhD Thesis, Yale University, New Haven.

Templeton A.R. & Levin D.A. (1979) Evolutionary consequences of seed pools. *American Naturalist*, **114**, 232–249.

Temprano M., Moreno I., Carmona M.J. & Serra M. (1994) Size and age at maturity of two strains of the rotifer *Brachionus plicatilis* in relation to food level. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **25**, 2327–2331.

Tsitsilas A. & Barry M.J. (2002) Optimising conditions for Australian *Daphnia* ephippia. *Verhandlungen Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **28**, 1460–1463.

Van der Linden A., Gadeyne J., Van Onckelen H., Van Laere A. & Declier W. (1991) Involvement of cyclic nucleotides in light –induced resumption of development of Artemia embryos. *Journal of Experimental Zoology*, **258**, 312–321.

Vandekerkhove J., Niessen B., Declerck S., Jeppesen E., Conde-Porcuna J.M., Brendonck L. & De Meester L. (2004) Hatching rate and success of isolated versus non-isolated zooplankton resting eggs. *Hydrobiologia*, **526**, 235–241.

Walker K.F. (1981) A synopsis of ecological information on the saline lake rotifer *Brachionus plicatilis* Müller 1786. *Hydrobiologia*, **81**, 159–167.

Wallace R. & Snell T.W. (1991) Rotifera. In: *Ecology and Classification of North American Freshwater Invertebrates* (Eds J.H. Thorp & A.P. Covich), pp. 187–240. Academic Press, New York.

Weider L.J., Lampert W., Wessels M., Colbourne J.K. & Limburg P. (1997) Long-term genetic shifts in microcrustacean egg bank associated with anthropogenic changes in lake Constance ecosystem. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **264**, 1613–1618.

(*Manuscript accepted 17 April 2006*)