

Assessing rotifer diapausing egg bank diversity and abundance in brackish temporary environments: an *ex situ* sediment incubation approach

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With 3 figures and 3 tables

Abstract: The hatching of diapausing eggs from sediment samples of ponds and lakes is a fast, easy, and economical technique proven useful for the assessment of diversity and abundance of zooplankton in permanent habitats. Here, we extend and optimize this approach for temporary brackish ponds which may experience great within-year variation in salinity conditions. We studied the species and numbers of rotifers emerging from the sediments of several brackish ponds and saline lakes from Eastern Spain incubated at the full range of salinity conditions observed at the study sites. On average, 82 % of the total observed species in weekly sampling for one year were detected in a single sample of both the sediments and water column. No statistical difference was found between both methods. However, some rotifer species were only observed in the water column samples, but not in sediment hatchlings, and *vice versa*. We concluded that both methods are not exhaustive by themselves but are complementary. Salinity affected the estimation of both rotifer richness and the density of individuals of each rotifer species that emerged from the incubated sediments. Differences in the density of emerging animals were also found among sites and among combinations of sites and salinities. The latter result indicates that in order to obtain an estimate of the emergence rate for each rotifer species present at a site, it is necessary to incubate sediments at salinity conditions close to their local values.

Key words: Diapause, emergence, rotifers, salinity, sediment incubation, temporary ponds.

Introduction

Zooplankton populations inhabiting temporally variable environments typically produce a dormant stage during their life cycle (Wiggins et al. 1980). These dormant stages (e.g., cysts or diapausing eggs) accumulate in the sediments of lakes and ponds forming demographic reservoirs (e.g., diapausing egg banks) that allow the survival of populations until favourable environmental conditions return. Furthermore, diapausing egg banks have been recognized to integrate the temporal variation

in the abundance and distribution of zooplankton organisms (Brendonck & De Meester 2003, Vandekerkhove et al. 2005a). This feature has been useful in diversity studies, since it is possible to compile an exhaustive inventory of the species present in a habitat and their abundance from a single set of sediment samples, instead of the repeated sampling of the water column during a whole growth season (May 1986, 1987, Dugan et al. 2002, Vandekerkhove et al. 2004, 2005b).

Different methods have traditionally been used to assess the abundance and viability of zooplankton

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dormant stages in the sediments of aquatic habitats, including: (1) flotation and isolation of diapausing eggs (Snell et al. 1983, Duggan et al. 2002, 2006), (2) direct egg counts (García-Roger et al. 2006), or (3) emergence estimation (De Stasio 1989, Wolf & Carvalho 1989, Cáceres 1998, Hairston et al. 2000). While the first two methods mentioned above imply sediment sampling, subsequent laboratory hatching experiments have to be performed in order to determine viability. The analysis of emergence is mainly carried out through *in situ* field emergence traps, but also *ex situ* sediment incubation as presented in this paper. This latter approach provides a direct estimation of the number of animals effectively contributing to population recruitment from the egg bank in the sediment (for review see Brendonck & De Meester 2003). Moreover, an emergence approach does not require a detailed knowledge of the large variety of dormant stage forms of different zooplankton taxa (Duggan et al. 2002, Bailey et al. 2003, Maia-Barbosa et al. 2003), or even a single taxonomic group like rotifers (Gilbert & Wurdak 1978, Pourriot & Snell 1983). An additional advantage of the *in situ* approach is that all natural hatching cues are applied, whereas laboratory incubations only mimic a part of the natural variation in hatching cues. However, emergence traps cannot be deployed at every site. For instance, deep sites may be inaccessible for scuba diving in order to install and check the traps, and remote-controlled approaches may be expensive to carry out (Clarke 2003). On the other hand, it may be also possible that the minimum design dimensions of emergence traps would exceed the maximum depth of extremely shallow ponds.

An easy alternative method to study the emergence of zooplankton species from the sediments in those sites where no traps can be deployed is laboratory sediment incubation (May 1986, 1987, Mnatsanakova & Polischuk 1996, Cáceres & Schwalbach 2001, Crispim & Watanabe 2001, Bell & Weithoff 2003). This approach often tries to experimentally mimic field conditions by using filtered water from the study sites and a set of temperatures that match the growing season range (May 1987, Duggan et al. 2002, Bell & Weithoff 2003). These studies have been typically restricted to permanent freshwater habitats of temperate regions, whereas temporary brackish water bodies from lower latitudes, which may suffer extreme within-year variation in salinity, have been rarely addressed (Nielsen et al. 2003). This is surprising since: i) due to their ephemeral nature, these environments are not always available for a classical limnological survey, ii) the salinity variation which organisms have to face in these

environments usually exceeds the temperature variation typical in freshwater habitats, and iii) saline waters are common on every continent and their water volume throughout the world is almost as great as the volume of fresh waters (Hammer 1986). In addition, salinity is widely recognized as a factor that influences rotifer life history characteristics and zooplankton community composition (Miracle 1974, Miracle & Serra 1989, Boronat et al. 2001). The change in salinity throughout a growth season may differentially affect the emergence of the species in a habitat, enhancing or limiting their ability to hatch, and shaping the (re)colonization dynamics after an adverse event in a pond (e.g., a drought and re-hydration cycle) or even in a species invasion process (Bailey et al. 2004, 2006, Duggan et al. 2006).

With this study we aim to optimize sediment incubation, a rapid and cost-effective technique for the assessment of zooplankton diversity in brackish temporary ponds, a little-known environment that indeed is not always available for water column sampling. First, we examine the effect of different salinity treatments on rotifer emergence (i.e., number of species emerged and abundance of each species) from laboratory incubated sediments of several saline lakes and brackish temporary ponds from Eastern Spain. Second, we check the list of emerging species obtained from this *ex situ* incubation approach with that from water column samplings of the sites studied as well as we perform a standardized comparison of both methods for the assessment of rotifer species richness. Finally, we test whether populations of a given rotifer species have different emergence responses to different salinities, correlated with the local salinity conditions of their sites.

Material and methods

Study sites

We sampled three brackish ponds and a saline lake from Eastern Spain that exhibit different patterns of water permanence (for further details, see García-Roger et al. 2006) and within-year salinity variation (Fig. 1).

Hondo Sur is the reduced vestige of an originally large lagoon in the Natural Park El Hondo de Elche, southeastern Spain. Currently, it is a small (0.2 km² area) and shallow (0.8 m average depth) brackish pond (ca. 15 g l⁻¹ annual average salinity) that has been described as semi-permanent since it typically has a lower water level during the summer but normally does not dry out completely. Salinity peaks during the minimum summer water level (Fig. 1).

Poza Sur is a very small pond (0.008 km² area) with less than 1 m of maximum depth located in the Prat de Cabanes-

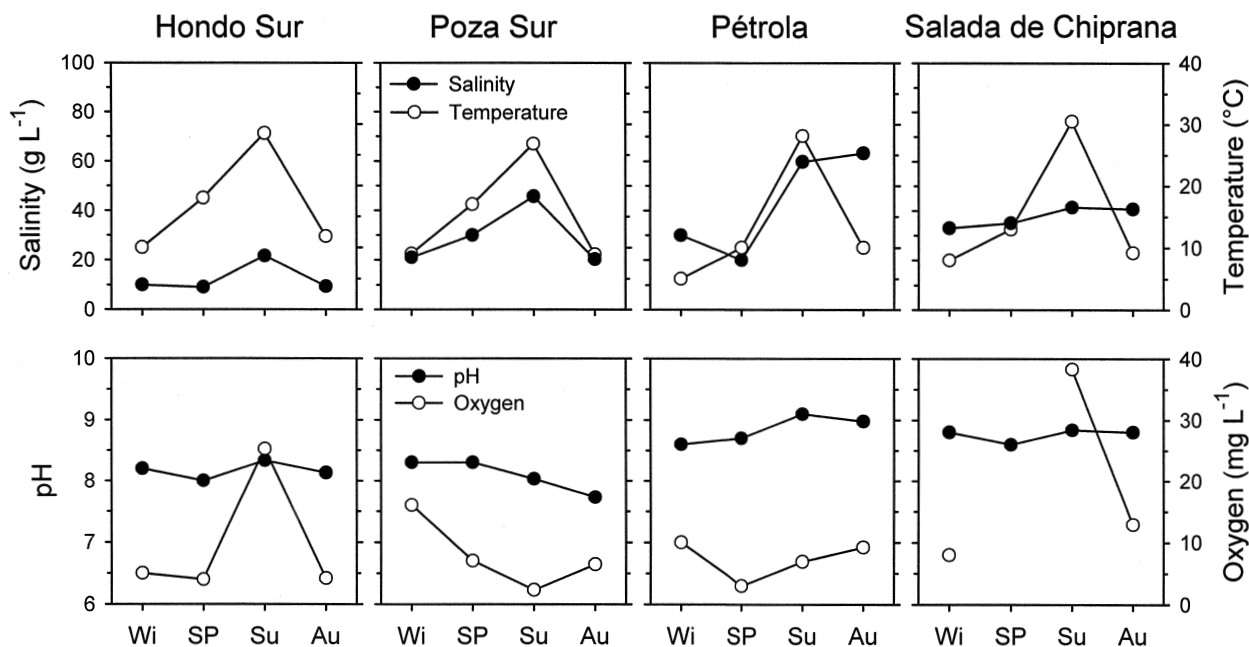


Fig. 1. Seasonal variation of the main physico-chemical parameters of the ponds and lakes studied during 2002.

Torreblanca Natural Park, a Mediterranean coastal wetland in eastern Spain. It is a seasonal pond that typically dries out in summer and refills in autumn showing a within-year pattern of salinity change similar to Hondo Sur (Fig. 1), although its annual average salinity is higher (ca. 35 g l⁻¹).

Pétrola is an endorheic pond belonging to the Júcar-Segura River Basin, southeastern Spain. It has a maximum refillable area of 1.74 km² and a maximum depth of just a few centimetres, with average annual salinity of 46 g l⁻¹. This pond typically dries out in summer, although it may remain partially dry for longer periods depending on the unpredictable incidence of rainfall in this semi-arid region (Rodríguez-Puebla et al. 1998). As shown in Fig. 1, evaporation of water in summer leads to a dramatic rise in salinity in this pond.

Salada de Chiprana (0.23 km² area), located in the Ebro River Basin, northeastern Spain, is a permanent, relatively deep (5 m average depth), hypersaline lake with an average annual salinity of 39 g l⁻¹. Because of its dimensions (i.e., size and depth) and ground water inflow, Salada de Chiprana hardly exhibits water level or salinity fluctuations (Fig. 1, see also Vidoondo et al. 1993).

Sediment sampling

Sediment samples were taken from a randomly-chosen, single sampling point in each one of the studied sites during summer 2003 at the end of the rotifer growth season, when the ponds and lake were dried out or exhibited low water level. Small variation in sediment rotifer diapausing egg abundance has been previously reported in the ponds studied (García-Roger et al. 2006). Sediment was collected using a Van Veen grab (2.5 dm² covered surface, Ejkelkamp Agrisearch Equipment) and transferred to 3 l plastic containers for transport to and storage in the laboratory. Samples were stored in the laboratory at 4 °C in the dark for 12 months in order to complete the refractory period of diapause (Hagiwara & Hino 1989).

Water column sampling

Water column zooplankton samples were taken at regular intervals of three months during 2002 from the sites studied in order to determine the species composition of each location. We restricted our study to rotifers, which were known to dominate the zooplankton in the study sites (Rodrigo et al. 2001, Lapesa 2004). Samples were taken by filtering 3–5 l of water through a 30-µm Nylal mesh, then fixed with 4 % formaldehyde and counted using an inverted microscope. Zooplankton species were identified using standard keys (Koste 1978, Segers 1995). This sampling design was used to qualitatively check whether the same species are found in the water column and the sediment. In order to check for the validity of our results, we compared our species list to that obtained in a previous, more intensive study of the ponds and lakes studied here (Lapesa 2004). The species list obtained from water column samples were in agreement with that previously reported from an intensive study by Lapesa (2004). Only one species from Poza Sur (*Notholca bipalium*) and two from Hondo Sur (*Keratella quadrata* and *Lepadella patella*) were not observed in our samples, but previously reported densities were lower than 0.5 ind l⁻¹. Additionally, when available, we took 16 l of water from each site to be used as culture media in sediment incubation experiments (see below). Water samples were pre-filtered through 200-µm and 30-µm sieve series to remove animals and finally filtered through GF/C filters (1 µm) to remove algae and protozoa. The water was stored frozen at -20 °C and in the dark until used.

Sediment incubation experiment

The top 4 cm of the sediment samples from each site, where viable diapausing eggs are more abundant (García-Roger et al. 2006), were carefully removed and thoroughly mixed. Then, 25 g (wet weight) subsamples of sediment were transferred to nine 250-ml Erlenmeyer flasks for each pond (4 ponds ×

9 flasks per pond = 36 flasks). A preliminary survey of the sediment samples of each pond through a direct egg-count procedure revealed that 25 g of sediment harbours at least 200 viable dormant stages of different zooplankton taxa. Prior to the incubation experiment, filtered water from each site was thawed and adjusted, either by dilution or evaporation, to three experimental salinities: 6 g l⁻¹, 18 g l⁻¹ and 36 g l⁻¹. Three replicates of each experimental salinity were tested for each site. The final volume in a flask (sediment + water) was 200 ml. The flasks were then incubated in a climate controlled room at 20 °C with a 12 : 12-h light/dark cycle (ca. 35 µE m⁻² s⁻¹, when illuminated). Daily, for up to 30 days of incubation, the water in each flask was carefully removed using a rotary peristaltic pump (Masterflex®, S/L™, Cole-Palmer®) and filtered through a 30-µm Nylal mesh to concentrate the emerged animals, which were later transferred to a plankton counting chamber to be counted under a stereomicroscope. When required, neonates were grown at 25 °C under constant illumination (ca. 35 µE m⁻² s⁻¹) and fed with *Tetraselmis suecica* (10⁵ cells ml⁻¹) in individual plates for further identification. Water in the flasks was renewed daily.

Statistical analyses

All statistics in this study were carried out using R 2.6.1 statistical software (Ihaka & Gentleman 1996). In order to compare rotifer species richness estimated from sediment emergence and water column sampling we followed the procedure by Vandekerckhove et al. (2005b) with minor changes. Accordingly, we first standardized both estimations of rotifer species richness by a measure of precision, given here by the inverse of sampling variance of individuals counted in each pond or lake by both two methods. Since the numbers of counted individuals differed among ponds and lake (range of individuals counted: 1–5100, average: 314 ± 147), we then normalized

rotifer species richness of each pond or lake by dividing by the total number of species retrieved by both methods combined. The overall comparison of rotifer species richness estimated between both methods was performed through a paired *t*-test (Sokal & Rohlf 1995).

A generalized linear model (GLM) on the species richness was used to test for an effect of salinity on the number of emerging species from each site. We assumed a Poisson distribution of data and log as link function (Nelder & Wedderburn 1972). Since multiple tests were performed, we used the Dunn-Šidák correction for statistical significance (Sokal & Rohlf 1995).

For each rotifer species, we used GLMs on the counts of emerging animals to test for differences in the emergence of a given rotifer species due to 'site' (i.e., the different ponds and lake studied), 'salinity', and 'site × salinity' interactions. A Poisson distribution of data and log as link function were assumed for the different models performed, and the Dunn-Šidák correction for the multiple tests was used.

Results

The complete list of rotifer species found in both the water column study and the sediment incubation experiment is shown in Table 1. A total of 18 rotifer taxa (17 identified monogonont species plus the rag bag of bdelloids) were found. Not all the species found in the water column emerged from the corresponding sediments. Conversely, some species were only observed in the sediment incubation experiment (*Lecane luna* in Pétrola and *Testudinella elliptica* in Hondo Sur). How-

Table 1. Rotifer species found in the water column and/or emerged from incubated sediments in the four sites studied (+: presence, -: absence).

Taxa	Hondo sur		Poza Sur		Pétrola		Salada de Chiprana	
	Water column	Sediment	Water column	Sediment	Water column	Sediment	Water column	Sediment
Bdelloidea	+	+	+	+	+	+	+	-
<i>Brachionus ibericus</i>	+	+	+	+	-	-	-	-
<i>Brachionus plicatilis</i>	+	+	+	+	+	+	+	+
<i>Brachionus rotundiformis</i>	-	-	+	+	-	-	-	-
<i>Colurella adriática</i>	+	-	+	-	-	-	-	-
<i>Colurella colurus</i>	+	-	+	-	-	-	-	-
<i>Encentrum</i> sp.	-	-	+	-	-	-	-	-
<i>Hexarthra fennica</i>	+	+	-	-	-	-	-	-
<i>Hexarthra oxyuris</i>	+	+	-	-	+	+	+	+
<i>Keratella quadrata</i>	-	-	-	-	+	-	-	-
<i>Lecane flexilis</i>	+	-	+	+	-	-	-	-
<i>Lecane grandis</i>	+	-	-	-	-	-	-	-
<i>Lecane hastata</i>	+	-	-	-	-	-	-	-
<i>Lecane inermis</i>	-	-	+	-	-	-	-	-
<i>Lecane luna</i>	-	-	-	-	-	+	-	-
<i>Notholca salina</i>	+	+	+	+	-	-	-	-
<i>Synchaeta cecilia</i>	+	+	+	+	-	-	-	-
<i>Testudinella elliptica</i>	-	+	+	+	-	-	-	-

ever, the overlap between both lists ranged from 66.7 % to 94.4 % (81.9 % on average). At the overall comparison, no significant statistical differences in rotifer species richness were found between both methods (Paired

t-test, *df* = 3, *t* = 1.358, *p* = 0.268). It is worthwhile to note that both sampling methods collected more species at coastal sites (Hondo Sur and Poza Sur) than at inland sites (Pétrola and Salada de Chiprana).

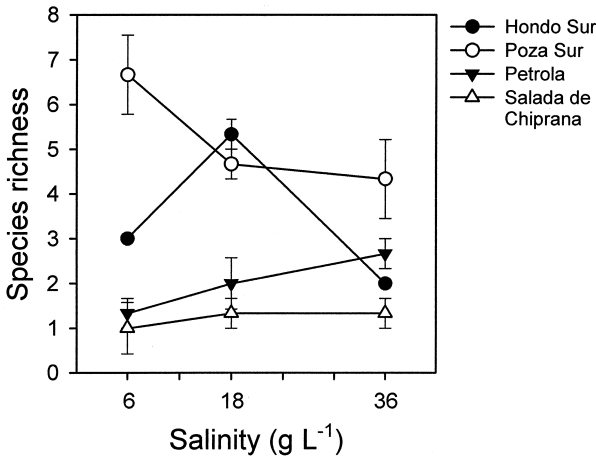


Fig. 2. Average rotifer species richness (\pm SE) in emergences from the sediments of the ponds and lake studied at the different salinity treatments.

Fig. 2 shows the average rotifer species richness for each pond or lake studied at the three experimental salinities. Rotifer species richness varied in relation to salinity at least in 3 out of 4 sites studied (Hondo Sur, Poza Sur and Pétrola), exhibiting quite different patterns. A statistically significant effect of salinity on rotifer richness was found only in Hondo Sur after Dunn-Šidák correction (Table 2). We found higher rotifer species richness at 18 g l⁻¹ in Hondo Sur than at any other salinity tested in the pond. The highest rotifer species richness in Poza Sur was found at the lowest salinity tested (6 g l⁻¹) and decreased with increasing salinity. In the case of Pétrola we found the opposite pattern – a decrease in rotifer species richness as salinity also decreases. Finally, in the case of Salada de Chiprana, whatever the salinity treatment rotifer species richness actually did not vary. In all

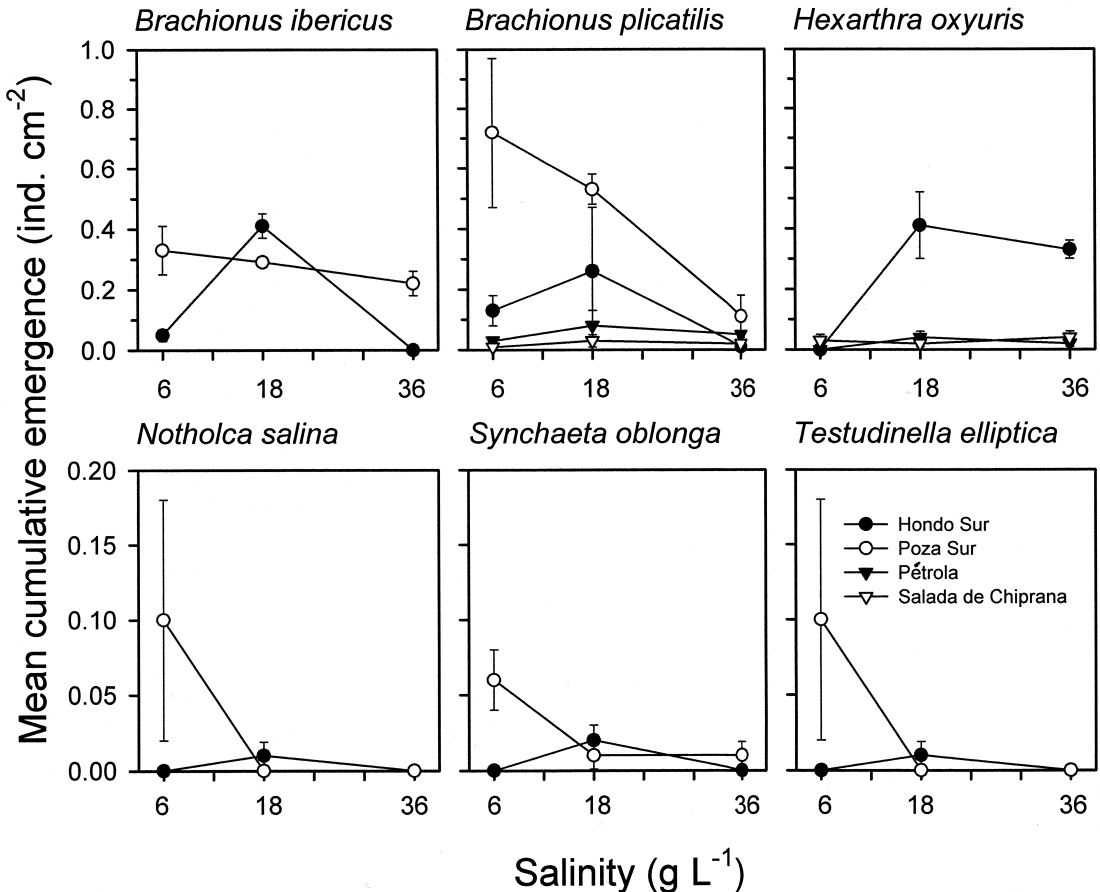


Fig. 3. Reaction norms of the emergence response (individuals cm⁻²) of different rotifer species from the ponds and lake studied to experimental salinity treatments. Only rotifer species found in more than one pond are presented. Values are means \pm SE.

the ponds and the lake studied, at least one salinity treatment included all the species emerged in the rest of treatments. Bdelloid taxa, likely including several species, were excluded from this analysis. Typically, sediment mass did not dramatically increase rotifer richness (data not shown).

Results of GLMs on the counts of emerging individuals of each species for the different effects studied are presented in Table 3. Not surprisingly, we found that sites differed statistically in the number of emerging individuals for almost every rotifer species. The emergence of 6 out of 10 rotifer species was significantly affected by salinity. Moreover, species response to salinity was dependent on the site of sediment origin (Table 3). The reaction norms relating emergence to incubation salinity for each rotifer species in the corresponding sites are showed in Fig. 3. Consistently, all rotifer species from Poza Sur reached their local

maximum emergence at the 6 g l⁻¹ salinity, whereas rotifers from Hondo Sur were more likely to emerge at the 18 g l⁻¹ salinity. Emergences in Pétrola and Salada de Chiprana were lower in general and did not exhibit a clear pattern.

Discussion

The hatching of diapausing eggs from pond and lake sediments has been recognized a very useful tool in zooplankton diversity studies and as a low-cost alternative to intensive water column sampling (Brendonck & De Meester 2003, Vandekerckhove et al. 2004). However, this approach has been used only rarely in saline environments (Nielsen et al 2003, Duggan et al. 2006). Nielsen et al. (2003) examined the effect of salinity on freshwater zooplankton emergence from diapause in habitats with small, episodic peaks in salinity (ca. 5 g l⁻¹). Zooplankton in temporary brackish or saline waters have to deal with important changes in ionic proportions as the water evaporates or refills the ponds (Williams 1987), and salinity could be expected to act as a species-specific cue for emergence from diapause. Along these lines, Duggan et al. (2006) have explored a broad range of salinities (0–30 g l⁻¹) to study the invasion risk by diapausing zooplankton coming from ship ballast sediments in marine and estuarine waters providing a valuable application of the method. In contrast, our study focuses for the first time on the emergence of zooplankton from natural sediments of temporary, saline continental waters as compared to water column sampling through a year. We explicitly

Table 2. Generalized linear models of the effect of the different salinity treatments on the estimations of rotifer species richness for each site.

Site	Salinity		
	<i>df.</i>	Deviance	<i>p</i>
Hondo Sur	2	15.28	< 0.001**
Poza Sur	2	7.63	0.022*
Pétrola	3	10.04	0.018*
Salada de Chiprana	2	0.00	1.000

* Significant at $p < 0.05$ without Dunn-Šidák correction for multiple tests.

** Significant at $p < 0.01$ with Dunn-Šidák correction for multiple tests.

Table 3. Generalized linear model analysis on the counts of emerging individuals of the different rotifer species. Dashes indicate species that were only present at one site according to previous data on species occurrence (Lapesa 2005), and, hence, were not included in the analysis of “site”.

Species	Site			Salinity			Site × Salinity		
	<i>df.</i>	Deviance	<i>p</i>	<i>df.</i>	Deviance	<i>p</i>	<i>df.</i>	Deviance	<i>p</i>
<i>Brachionus ibericus</i>	1	0.09	0.762	2	37.09	< 0.0001**	2	34.53	< 0.0001**
<i>B. plicatilis</i>	3	209.06	< 0.0001**	2	72.40	< 0.0001**	6	17.27	0.01*
<i>B. rotundiformis</i>	–	–	–	2	5.04	0.081	–	–	–
<i>Hexarthra fennica</i>	–	–	–	2	5.55	0.063	–	–	–
<i>H. oxyuris</i>	2	101.98	< 0.0001**	2	40.63	< 0.0001**	4	29.67	< 0.0001**
<i>Lecane flexilis</i>	–	–	–	2	6.53	0.038*	–	–	–
<i>L. luna</i>	–	–	–	2	2.20	0.333	–	–	–
<i>Notholca salina</i>	1	9.75	0.002**	2	19.48	< 0.0001**	2	6.88	0.032*
<i>Synchaeta cecilia</i>	1	3.85	0.049	2	4.01	0.134	2	6.19	0.045*
<i>Testudinella elliptica</i>	1	9.75	0.002**	2	19.48	< 0.0001**	2	6.88	0.032*

* Significant at $p < 0.05$ without Dunn-Šidák correction for multiple tests.

** Significant at $p < 0.005$ with Dunn-Šidák correction for multiple tests.

explored the effect of a wide range of hatching salinities (6–36 g l⁻¹), which occur naturally in saline lakes and temporary ponds from semi-arid regions (Comín & Alonso 1988, Rodríguez-Puebla et al. 1998). Our study also provides criteria to optimize the incubation conditions in these understudied habitats.

Similarly to previous studies addressing the suitability of a sediment incubation approach versus intensive water column sampling campaigns (Vandekerckhove et al. 2005b), the overlap in the rotifer species list obtained by both methods was not complete, but it was ca. 80 % on average. Although no statistical difference has been found between the two methods, a result that initially supports sediment incubation as a fast, effective and reliable tool for biodiversity studies, we stress that, to be fair, differences between both methods demand further discussion.

The majority of the non-overlaps were due to species that were found only in the water column samples but did not emerge from the sediments. Several explanations may account for this result: 1) low sediment sample size, 2) unsuitability of hatching conditions, and 3) unexpected long times of obligate diapause for some species.

The discrepancy between the sediment incubation approach and the water column sampling study could be due to a sampling effect if the diapausing eggs of the non-emerging species were scarce in the sediments or had a patchy spatial distribution (Carvalho & Wolf 1989, García-Roger et al. 2006). Interestingly, the species that were not observed in the sediment incubation experiment were also rare in the water column (less than 6 ind l⁻¹, data not shown), and so, likely had low-density banks. As a consequence of demographic stochasticity, diapausing eggs of rare species may not receive the signal for hatching due to burial and not be observed (Marcus & Schmidt-Gengenbach 1985). Excluding our first-ever record of the freshwater rotifer *K. quadrata* in Pétrola, all the species undetected in the sediment have been previously observed in water samples from the sites studied (Rodrigo et al. 2001, Lapesa 2004). This suggests that these rare species likely are not recent or episodic colonizers without a diapausing egg bank. Furthermore, the lack of marked habitat preferences (e.g., littoral, pelagic or benthic) of these species suggests that patchy spatial distributions are unlikely to affect their egg banks. In short, although an intensive sampling of the sediment may increase the number of species detected, our results suggest that exploring different incubation conditions is more useful for biodiversity studies in temporary, varying habitats (see Fig. 2).

Hatching conditions assayed in the laboratory could be inadequate for a given species, which would also explain the lack of correspondence between both methods pointed above. In this sense, we have explored a range of salinities that covers the whole within-year variation in salinity of the sites studied. Our expectation was that the incubation of sediment samples at different salinities would greatly improve rotifer richness. We observed that specific treatments worked much better than others with respect to the numbers of species emerged. This was especially true for Hondo Sur, where at 18 g l⁻¹ salinity we found the highest rotifer richness. Temperature, as found in freshwater habitat studies, might also affect rotifer emergence (May 1987, Duggan et al. 2002, Bell & Weithoff 2003). However, temperature is unlikely to be an important factor in the emergence of rotifers from the sediments studied here. First, temperature variation is lower than in other ponds and lakes studied from other latitudes. Moreover, note that even winter species (e.g., *B. plicatilis* and *N. salina*) hatched at our fixed experimental temperature of 20 °C. Following Pourriot & Snell (1983) and May (1987), this temperature is found within the thermal preference of the whole set of populations studied. Most likely, the effect of temperature is to increase the metabolic rates, the developmental rates and consequently to shorten the time to hatch, rather than to act as an inducing signal (Pourriot et al. 1980).

It is possible that diapausing eggs of non-emerged species may need a longer time of obligate diapause before hatching (Pourriot & Snell 1983) or longer periods of embryo development after the breaking of dormancy (e.g., copepods may need >4 weeks in order to emerge, Teasdale et al. 2004). Note also that salinity may influence the respiration rate, and thus the metabolism of diapausing embryos, as less oxygen can be dissolved in saline water (Williams 1987). However, with only one exception (*L. flexilis* in Poza Sur), we found that the emergence curves for all rotifer species, whatever the salinity treatment and the site of origin, saturated after 30 days of incubation (data not shown). Hence, more hatchlings for the emerged species were not expected. Information on the refractory period of diapause is restricted to *B. plicatilis* (i.e., 1 month, accordingly to Hagiwara & Hino 1989), but it is very unlikely that refractory periods longer than 12 months could evolve, as suitable environmental conditions are expected to return annually.

Some species were only detected after the incubation of the sediments, but not in the water column samples. We have no knowledge of any particular habitat

preference for these species, so failure to sample the water in particular habitats is unlikely to explain this observation (Vandekerkhove et al. 2005b). A possible explanation for our finding is based on among-year variation in the water column rotifer assemblages. Species absent in a particular year could be present in the surficial sediment layer, as it integrates diapausing egg production over several years (Hairston et al. 1999). Of course, ephemeral populations in the water column might remain undetected. In summary, our result shows that both methods are complementary (see also Vandekerkhove et al. 2005b).

Several studies have demonstrated that, in comparison to the incubation of complete sediments, the isolation of diapausing eggs before incubation usually allows the extraction of a higher number of taxa and individuals (Engel & Hirche 2004, Bailey et al. 2005, Vandekerkhove et al. 2004, Duggan et al. 2006, see García-Roger et al. 2006 for comparison in the *Brachionus plicatilis* species complex). This illustrates that it is not possible to estimate the size of diapausing egg banks in the sediment from the numbers of emerging animals from incubated sediments. Nevertheless, when choosing a method (or methods) for zooplankton biodiversity assessments some other logistic reasons could be involved and, an *ex situ* incubation approach offers a low-cost and fast, complementary alternative method (May 1986, Duggan et al. 2002, Vandekerkhove et al. 2005b).

Our study shows that ecological conditions in temporary brackish habitats should be considered for the optimization of the *ex situ* sediment incubation technique. Rotifer species responded differentially to salinity treatments. Thus, different salinity treatments covering the natural range of salinities at the sites should be used for characterizing the emergence of different species. Moreover, we found that the response of a particular rotifer species to salinity varied among sites, which may be an indication of local adaptation to salinity conditions in the ponds studied. Interestingly, we observed that rotifer species from Hondo Sur, a permanent pond with rather stable salinity, showed maximum emergence densities at 18 g L⁻¹ salinity, which indeed is close to its annual average salinity. In contrast, we observed that winter species in Poza Sur (*B. plicatilis*, *N. salina*, *S. oblonga*) reached their maximum emergence densities at the lowest salinity treatment (6 g L⁻¹ salinity), which reflects the salinity conditions that typically occur in Poza Sur during winter. This means that, in order to obtain an estimate of the emergence density for each rotifer species in a particular site, we should incubate the sediment at

those conditions closer to their local preference ones. This implies the seasonal collection of at least physico-chemical data.

The *ex-situ* sediment incubation technique is especially useful for studying remote and unpredictable habitats. This tool also could be improved with knowledge of the ecology of planktonic species. These data should consider the challenging problem of the existence of cryptic species complexes (Knowlton 1993, Gómez et al. 2000, 2002, Ciroso-Pérez et al. 2001, Ortells et al. 2003, Campillo et al. 2005), and incorporate information on diapause patterns of the species. Experiments on the environmental factors inducing diapause would be very useful in this context.

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