

## Uncovering hidden species: hatching diapausing eggs for the analysis of cladoceran species richness

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### Abstract

Effective methods for the detection of species are highly needed in biodiversity research. The assessment of richness is especially difficult for short-lived aquatic organisms, like plankton. Because of the high degree of spatial and temporal variation in plankton community composition, the compilation of complete plankton species lists requires intensive sampling programs. Many large-scale studies on planktonic organisms are based on single occasion samplings and inevitably fail to detect a substantial fraction of the species. In the present study, we assessed species richness of Cladocera (Branchiopoda, Crustacea) in 88 European lakes by repeated sampling of the active communities and by an alternative method based on the hatching of diapausing eggs from the lake sediments. Identification of hatchlings obtained from egg bank samples enabled the detection of about twice as many cladoceran species than identification of an equal number of individuals retrieved from active community samples. Loglinear analyses revealed a strong species by method interaction, suggesting that both methods are highly complementary and should preferably be applied in combination. Some species were underrepresented in the active community samples, probably because of the lack of spatial and temporal integration in these samples. Other species were underrepresented in the hatching assemblages as a result of species-specific propensities to produce diapausing eggs or hatch from such eggs.

The simplicity of the definition of species richness, being the number of species present at a site, contrasts with the complexity of its measurement (reviewed in Gotelli and Colwell 2001; Magurran 2003). Richness assessments are particularly difficult for short-lived organisms, such as zooplankton (Arnott et al. 1999). The community dynamics of short-lived organisms are very variable, both in space (Sommer et al. 1986; Grover 1999) and time (De Meester et al. 1999; Burks et al. 2002). Due to this spatial and temporal heterogeneity,

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assessments of their species richness are only deemed reliable if based on representative year-round collections of samples taken at a large number of locations.

There is a clear need for tools that allow a rapid, cost-effective, and reliable assessment of species richness in short-lived organisms. Such tools should facilitate the detection of extant patterns of ecosystem diversity and thus contribute to a better understanding of diversity, its relation with its drivers, and with ecosystem functioning, stability, and resilience (Loreau 2000; Lyons and Schwartz 2001). They may also contribute to the evaluation of the relative importance of local versus regional factors as determinants of diversity (Shurin et al. 2000; Cottenie et al. 2003). Furthermore, they may help to identify sites that deserve prioritization for conservation.

Many groups of short-lived aquatic organisms produce long-lived diapausing eggs (overview in Cáceres 1997; Brendonck et al. 1998). The horizontal and vertical distribution of diapausing eggs in the sediments of ponds and lakes tends to be homogenized by wind-induced resuspension, the activity

**Table 1.** Overview of studies that assessed species richness through analysis of hatchling assemblages (EB method) and through analysis of active community samples (AC method)

	May 1986	Havel et al. 2000	Crispim and Watanabe 2001	Duggan et al. 2002	Vandekerkhove et al. 2005a	This study
Country	Great Britain	United States	Brazil	New Zealand	Belgium	Denmark, Belgium, The Netherlands, and Spain
Habitat type	Loch (depth: 25 m)	Riparian wetlands	Reservoir (depth: 15 m)	Deep lakes	Shallow lake	Shallow lakes
Number of water bodies	1	12	1	2	1	88
Taxonomic group	Rotifera	Macrozooplankton	Cladocera	Rotifera	Cladocera	Cladocera
EB method						
Number of sampling locations	6	10	10	1	12	10
Number of sampling occasions	1	1	1	1	1	1
Sampling period	Feb. 1984	1994-1995 (Oct. or July)	March 1997	Jan. 1999	April 2000	2001 (March or Oct.)
AC method						
Number of sampling locations	*	*	*	1	8	8 (<5 ha) or 16 (>5 ha)
Number of sampling occasions	162+	2-72	3	4	6	6
Sampling period	1977-1982	1994-1997 (May-Oct.)	Dec. 1995, Feb. and April 1996	March 1997-April 1998	1996-2000 (May-Oct.)	2000 or 2001 (May-Oct.)

\*Information is not available.

of bottom-dwelling organisms, and sediment focusing (i.e., the lateral transport of particles along the slope) (Douglas and Rippey 2002; Vandekerkhove et al. 2005a). This characteristic of egg banks provides promising opportunities for the development of methods that allow the assessment of community structure and diversity integrated over time and space (Hairston 1996; Jeppesen et al. 2003; Brendonck and De Meester 2003). The analyses of egg banks may be relatively cost efficient because sediments can be collected in one single sampling campaign. In contrast, active communities have to be repeatedly sampled at a large number of different locations. Species richness assessments based on the analysis of egg bank samples could, in principle, involve morphological identification of diapausing eggs (Vandekerkhove et al. 2004a). However, knowledge about the morphology of diapausing eggs is scarce or lacking for many taxa. Therefore, species identifications of diapausing eggs most often require incubation under conditions suitable for hatching and subsequent identification of fully grown hatchlings.

So far, the empirical data are too fragmentary to allow a general evaluation of the hatching method: the number of studies that make a direct comparison between the richness observed in egg banks and active community samples is still

very limited (Table 1). The methodologies and procedures followed in these studies differ widely. For example, studies differ in the timing of sampling, the sampling device, the core depth, the storage temperature, and the hatching condition(s) applied. With the exception of Havel et al. (2000), the studies are based on only one or two lakes, and they are all confined to a single geographic region. Furthermore, the active community data of most studies are based on a low number of samples (during time and in space; Table 1).

With this study, we aimed to make a thorough and standardized evaluation of the hatching of diapausing eggs as an effective method for the assessment of species richness in cladoceran populations of shallow lakes. Our evaluation is based on a comparison of the number and identity of species detected in cladoceran hatchling assemblages with samples derived from the active communities. This study presents for the first time data on egg bank richness that are based on a large number ( $n = 88$ ) of shallow lakes from three different geographic regions (Denmark, Belgium/The Netherlands, and South of Spain) that were selected along broad gradients of productivity, connectivity, surface area, and macrophyte cover. Diapausing eggs were hatched according to a standardized and optimized protocol (Vandekerkhove et al. 2005b),

while the assessment of the active communities was based on a very extensive sampling effort.

### Materials and procedures

**Sampling**—We collected samples of both the active zooplankton communities and the egg banks of 95 shallow European lakes (average depth < 3 m). To attain a broad scope, lakes were selected in three different European regions: Denmark (29 lakes), Belgium/The Netherlands (34 lakes), and the south of Spain (32 lakes). The lakes were not selected randomly: to incorporate a large variety of shallow lake types, the lakes were selected along broad gradients of surface area, productivity, water plant cover, and degree of isolation (for more information, see Declerck et al. 2005).

**Egg bank.** In Denmark and Belgium/The Netherlands, sediment samples were collected in March 2001, before the onset of the zooplankton growing season (Herzig 1985; Hairston et al. 2000). In Spain, weather conditions are favorable year-round for reproduction, although many lakes dry out during the second half of summer. The egg banks of the Spanish lakes were sampled during October 2001, the period preceding the onset of the new growing season in the temporary lakes. Sediment samples were collected by means of a hand-operated sediment core sampler that is composed of a 6-m long shaft and a 50-cm long acrylic tube (diameter: 5.2 cm), which is screwed into a cylinder with a built-in silicone contra flap. Each lake was sampled at 10 locations, five were randomly selected in the littoral and another five were randomly selected in the open water area. Only the upper 3 cm of each core were retained (corresponding to approximately 100 g wet weight per sample), covering typically less than 10 y in these types of shallow lakes (Søndergaard et al. 2003). Immediately after collection, samples were wrapped in aluminum foil and transported to the laboratory in a cool box.

**Active zooplankton community.** The active zooplankton communities of the lakes were sampled with a tube sampler. This device essentially consists of a 2-m long Plexiglas tube, 75 mm inner diameter, with a built-in contra-flap that can be closed at the desired depth by pulling an attached rope. Samples were taken monthly during the growing season (May–October) of either 2000 or 2001. Depending on the surface area of the lake, depth-integrated samples of 6 L or 3 L were taken at 8 (<5 ha) or 16 (>5 ha) locations, respectively. The locations were randomly assigned on a grid covering both the littoral and pelagic zone. The samples of the different locations were pooled and the resulting sample was then thoroughly stirred and quantitatively subsampled (15 L) for zooplankton. Cladocera of the subsample were concentrated with a plankton net (mesh size: 64  $\mu\text{m}$ ).

**Sample analysis**—Egg bank. After a pre-incubation period of approximately 1 y in the dark at 4°C (earlier analyses indicated that 6 weeks suffice; Vandekerkhove unpubl. data unref.), the pelagic and littoral sediment samples were pooled per lake. Subsequently, diapausing eggs were isolated from subsamples

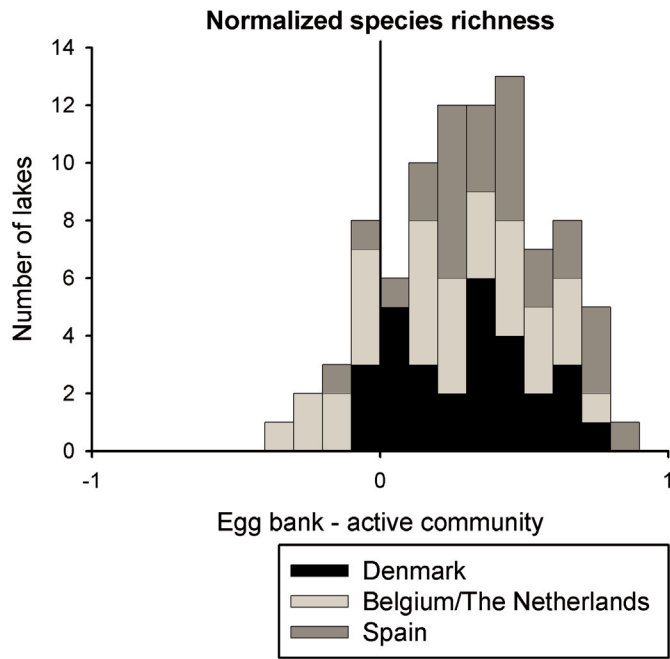
(ca. 100 g per sample) by means of the sugar flotation method developed by Onbé (1978) and modified by Marcus (1990). Isolation of diapausing eggs facilitates rapid and synchronized hatching (Vandekerkhove et al. 2004b). Isolated diapausing eggs were incubated in 2-L aquaria filled with diluted ADAM medium (200  $\mu\text{S cm}^{-1}$ , Kluttgen et al. 1994) at simulated spring conditions (15°C, long day photoperiod: 16 hour light day<sup>-1</sup>; Vandekerkhove et al. 2005b). Additional subsamples were processed until 100 hatchlings were obtained or until all subsamples were used. The total amount of sediment used therefore depended on the number of hatchlings (average wet weight of sediment used: 337 g; range: 85–1098 g). Cladoceran hatchlings were removed at 3-d intervals during a period of 30 d, identified to genus (Daphniidae: *Daphnia*, *Ceriodaphnia*, *Scapholeberis*, *Simocephalus*; Bosminidae: *Bosmina* and Leptodoridae: *Leptodora*) or family level (Sididae, Chydoridae, Macrothricidae and Moinidae), and transferred to 50 mL jars filled with filtered pond water (mesh size: 30  $\mu\text{m}$ ). Hatchlings from easy-to-grow taxa were further fed with *Scenedesmus obliquus* (100,000 cells/mL). Once these hatchlings reached a morphologically identifiable stage, they were preserved and identified to species level using the key of Flößner (2000). Because of mortality, a limited fraction of hatchlings (approximately 15%) could only be identified to genus or family level. These hatchlings were proportionally assigned to the species from the same genus or family that could be detected from fully grown individuals. Hatchlings of taxa that were difficult to grow were identified to genus level immediately after hatching and not grown (*Bosmina*, *Diaphanosoma*, *Ilyocryptus*, *Leptodora*, *Moina*, *Macrothrix*, and *Polyphemus*).

**Active zooplankton community.** At the end of the 6-month sampling period, equal subvolumes of the monthly samples were merged to form one pooled sample for each lake. This pooled sample thus integrated spatial (littoral and pelagic) and seasonal variation (May–October, six sampling occasions) in cladoceran community composition. The sample was screened for 300 cladocerans, using the same taxonomic resolution and key as described for the analysis of hatchling assemblages.

We evaluated the hatching method by comparing the species richness and species identity of the hatchling assemblages with that of active community samples. A total of 95 lakes were visited for sampling. In one lake, a thick layer of plant material hampered the collection of sediment. Six other lakes were excluded from further analysis because the numbers of animals retrieved from the active community sample (2 lakes) or the sediment sample (4 lakes) were too low ( $n < 10$ ).

### Assessment

**Species richness**—Cladoceran species richness was determined from both the active community sample and the hatchling assemblage. To allow the comparison of richness data between both methods, a standard number of cladoceran



**Fig. 1.** Frequency distribution of normalized species richness for 88 lakes based on the difference between the normalized species richness of the hatchling assemblages and the normalized active community species richness. Lakes were situated in three European regions.

individuals was identified for each of both methods. For each lake, this standard number was defined as the lowest number of individuals retrieved by one of the two methods. This number varied considerably between lakes (range of individuals retrieved: 10-300; average:  $118 \pm 16$ ).

For the majority of lakes (74 of 88 lakes), standardized species richness in the hatchling assemblages was higher than in the pooled active community samples (number of species:  $7.9 \pm 0.8 > 5.1 \pm 0.6$ ). Because the standard number of investigated individuals differed between lakes, an overall comparison required normalization of the richness data per lake. For each method-lake combination, this was done by dividing the number of species revealed by the method by the total number of species that was revealed by both methods combined. From Fig. 1, it is clear that in the majority of the lakes hatchling assemblages contained a higher fraction of the species. For a given number of identified Cladocera, species numbers obtained through analysis of hatchling assemblages were on average double as high as the numbers of species that were counted in the corresponding active community samples (paired *t* test over regions: *df* = 87, *t* = 10.2, *P* < 0.0001; Table 2). This difference in species richness between both methods was similar in the three geographic regions (1-way ANOVA: *df* = 2, *F* = 1.9, *P* > 0.05; Table 2). The probability of hatching a species from the sediment samples of a particular lake upon detection in the corresponding active community sample was on aver-

**Table 2.** Ratio of the species richness of the hatchling assemblage on the species richness recorded for the active community sample after standardization to a fixed number of identified individuals ( $Z \pm 2 \times SE$ )\*

Region	<i>t</i> Test results		
	$Z \pm 2 \times SE$	<i>df</i> †	<i>t</i> value
Denmark	$1.8 \pm 0.3$	28	6.8**
Belgium/The Netherlands	$1.7 \pm 0.3$	31	4.2**
Spain	$2.2 \pm 0.5$	26	7.7**

\*The average *Z* values are separately given for each of the studied geographic regions. For each geographic region, paired *t* tests were applied to test for differences between the species richness of hatchling assemblages and active community samples. For details, see text.

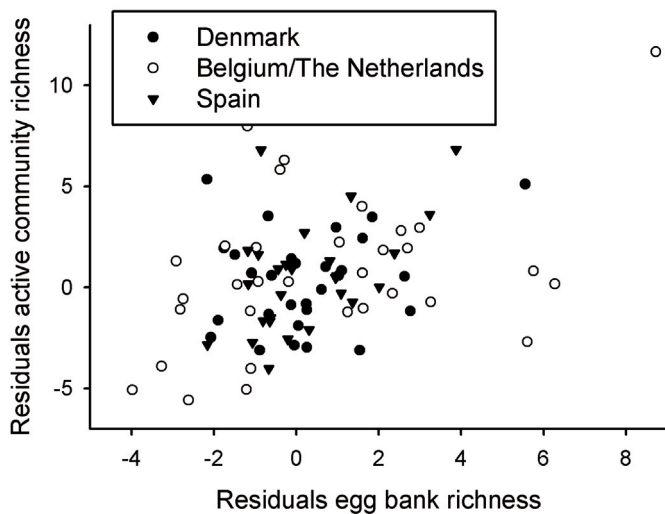
†*df*, degrees of freedom.

\*\**P*-value of *R* < 0.01.

age 0.61 (Denmark: 0.59; Belgium/The Netherlands: 0.59; and Spain: 0.69). This probability was substantially lower in the reverse case (average over regions: 0.47; Denmark: 0.45; Belgium/The Netherlands: 0.51; and Spain: 0.42).

To assess the degree of association between the species numbers detected by the two methods, species richness was first corrected for the number of investigated individuals. The relationship between species richness and number of individuals is generally described as  $S = k \times I^x$  (Siemann et al. 1996; Hillebrand et al. 2001), where *S* is species richness, *I* is the number of identified individuals, and *k* and *x* are coefficients. The coefficients of the equation can be estimated by linear regression of the logarithmically transformed *S* on the logarithmically transformed *I*. Using the equation, we calculated deviations between expected and observed richness values (residuals). This was separately done for both methods in each of the three investigated geographic regions. Finally, per geographic region, we calculated the correlation coefficient between the residuals obtained for both methods. These correlations were significant for the regions Belgium/The Netherlands (*r* = 0.41, *P* = 0.019) and Spain (*r* = 0.50, *P* = 0.008), but not for Denmark (*r* = 0.22, *P* > 0.05; Fig. 2).

**Species identity**—The methods showed systematic differences in their effectiveness to reveal species, and this effectiveness depended on species identity. Loglinear analysis applied on the occurrence data of the 20 commonest species in all regions combined revealed an overall species  $\times$  method interaction (*df* = 19,  $\chi^2$  = 98.4, *P* < 0.0001). Effectivity differences between methods were further explored for individual species with Fisher exact tests (Table 3). For 11 of the 20 commonest species, detection frequency was significantly higher with the hatching method than through analysis of the active community sample. The reverse was true for six species (Fisher exact tests: *P* < 0.05; Table 3). These results do not only underscore the potential of the hatching method to assess cladoceran species richness, they also indicate that both methods are highly complementary.



**Fig. 2.** Species richness as obtained through incubation of egg bank samples and through analysis of pooled active community samples, following correction for the number of examined individuals. Corrections were done by taking the residuals of the observed species richness and the richness that was expected based on the region specific relationship between species richness and number of identified individuals (see text). A standardized number of individuals was identified per lake for both methods. The data were obtained for 88 shallow lakes from three geographic regions in Europe.

## Discussion

The results of this first large-scale methodological evaluation to assess zooplankton species richness advocate hatching of diapausing eggs as an effective method for the detection of species in zooplankton communities. Indeed, identification of hatchlings obtained through the incubation of egg bank samples enabled, on average, the detection of about twice the number of cladoceran species compared to identification of an equal number of individuals in active community samples. Nevertheless, a comparative analysis of species lists obtained by both methods indicates that the detection probability of specific species differs between the two methods. This is probably due to a failure of both methods to yield complete species lists.

The absence of some species in the active community samples may be the result of habitat-specific capture efficiency of the sampling method and the difficulty to integrate spatiotemporal heterogeneity in active cladoceran communities. It is known that species living in close association with substrates, like benthic- or macrophyte-associated species, are less efficiently captured by zooplankton sampling devices than open water species (De Stasio 1993, Wetzel and Likens 2000). Although we used a tube sampler that enables the collection of benthic and macrophyte associated species, capture efficiency of the sampling device may still have been relatively low for taxa like *Ilyocryptus* sp., *Alona* sp., *Leydigia* sp., and *Pleuroxus* sp. As a consequence, these taxa were then more easily detected with the hatching

method than through analysis of active community samples. However, the observation that also open water taxa like *Daphnia pulex* and *Diaphanosoma brachyurum* were more frequently detected through the hatching method indicates that a reduced capture efficiency may not be the only factor responsible for the shorter species lists obtained through analysis of active community samples. Possibly, some additional species were found in the egg bank samples because of the lack of integration of inter-year variation in the active community samples (Vandekerkhove et al. 2005a). Indeed, the egg bank samples covered a period of several years, whereas the active community samples were collected at six different occasions within the same growing season.

There were also a few species that were exclusively detected in the active community samples (Table 3). Some of these species were possibly underrepresented in the hatchling assemblages because of low diapausing egg production in the corresponding field population. Indeed, not all populations contribute to the egg bank build-up in equal proportions (Jankowski and Straile 2003; Tessier and Cáceres 2004). We may also have failed to hatch some species from the sediments because of the limited viability of their diapausing eggs. While most taxa produce diapausing eggs that remain viable for several years or even decades (Hairston et al. 1995), it has been reported that diapausing eggs of *Leptodora* stored during a period of 1 year or longer do not hatch successfully (Herzig 1985). Finally, a number of species may have been missed because all diapausing eggs were incubated under a single condition (one photoperiod and temperature combination). Conditions for optimal hatching are known to vary among cladoceran taxa (Schwartz and Hebert 1987; Cáceres 1998; Vandekerkhove et al. 2005b). As a result, the species list of hatchling assemblages may, to some extent, be biased toward taxa that best hatched under the incubation conditions chosen for this study.

Although there is some association in species numbers obtained by both methods, the presence of a species by method interaction in the loglinear analysis suggests that the joint application of both methods will yield the most complete picture of the number and identity of taxa present in a water body. Budgetary and/or time limitations may force researchers to choose for one single method. In this case, our results suggest that application of the hatching method is the better choice for the assessment of cladoceran richness. Analysis of egg bank samples allows faster detection of a higher number of species than analysis of active community samples. The hatchling assemblages include pelagic, littoral, and benthic species and cover the richness of several years (Vandekerkhove et al. 2005a). As such, analyses of egg bank samples provide a more complete and integrated picture of the richness of a lake compared to analyses of active community samples. In addition, the hatching method will in most cases also be the most cost efficient, because it only requires a single sampling event. This is especially important when the studied

**Table 3.** Frequency of detection for all cladoceran species encountered in this study\*

Species	HP†	N‡	AC method			EB method		
			DK	BNL	SP	DK	BNL	SP
<i>Bosmina</i> sp.	P	69	28	30	9	25	30	1
<i>Daphnia galeata/cucullata</i>	P	59	24	21	5	21	26	7
<i>Ceriodaphnia pulchella</i>	L+P	57	19	25	0	27	28	1
<i>Alona rectangula</i>	L	55	4	6	8	19	18	16
<i>Diaphanosoma brachyurum</i>	P	54	14	5	11	17	19	15
<i>Chydorus sphaericus</i>	L	54	20	23	7	3	8	3
<i>Daphnia magna</i>	P	34	4	5	9	5	8	15
<i>Simocephalus vetulus</i>	L	33	5	11	4	5	11	7
<i>Ilyocryptus</i> sp.	B	27	2	3	0	8	12	5
<i>Sida cristallina</i>	L	27	1	12	0	11	5	1
<i>Ceriodaphnia reticulata</i>	L+P	27	1	3	17	3	1	20
<i>Pleuroxus aduncus</i>	L	24	2	6	2	2	10	5
<i>Ceriodaphnia laticaudata</i>	L+P	22	0	0	2	5	6	11
<i>Alona affinis</i>	L	22	3	4	0	11	6	1
<i>Alona quadrangularis</i>	L	22	10	4	0	12	3	0
<i>Ceriodaphnia quadrangula</i>	L+P	21	0	5	1	4	9	7
<i>Acroperus harpae</i>	L	21	5	13	1	5	6	0
<i>Leydigia acanthocercoides</i>	L	20	0	0	2	4	8	8
<i>Disparalona rostrata</i>	L	19	0	8	0	4	12	0
<i>Scapholeberis mucronata</i>	P	17	2	7	0	3	9	0
<i>Daphnia pulex</i>	P	16	0	3	0	2	10	4
<i>Pleuroxus uncinatus</i>	L	16	3	1	0	13	2	0
<i>Leptodora kindti</i>	P	16	11	5	0	0	1	0
<i>Daphnia parvula</i>	P	15	0	8	0	0	14	0
<i>Moina</i> sp.	P	15	0	1	7	1	1	10
<i>Dunhevedia crassa</i>	L	14	0	0	7	0	0	14
<i>Alonella nana</i>	L	14	6	7	0	1	4	0
<i>Daphnia ambigua</i>	P	13	0	6	0	0	13	0
<i>Leydigia leydigi</i>	L	12	0	1	0	2	4	5
<i>Eurycercus lamellatus</i>	L	11	2	8	0	1	0	0
<i>Macrothrix</i> sp.	B	10	0	1	4	1	1	7
<i>Graptoleberis testudinaria</i>	L	10	1	6	0	2	3	0
<i>Ceriodaphnia dubia</i>	L+P	8	0	5	0	0	7	1
<i>Oxyurella tenuicaudis</i>	L	7	0	0	2	1	1	4
<i>Tretocephala ambigua</i>	L	7	0	0	3	0	0	6
<i>Polyphemus pediculus</i>	P	7	2	5	0	0	0	0
<i>Ceriodaphnia megops</i>	L+P	6	0	0	0	1	5	0
<i>Alona guttata</i>	L	6	2	3	0	0	2	0

Continued

**Table 3**—Continued

<i>Pleuroxus trigonellus</i>	L	5	1	0	0	3	1	0
<i>Alona costata</i>	L	5	0	1	0	3	1	0
<i>Pleuroxus truncatus</i>	L	5	1	3	1	0	0	0
<i>Picripleuroxus laevis</i>	L	4	1	3	0	0	1	0
<i>Alona salina</i>	L	3	0	0	3	0	0	1
<i>Daphnia similis</i>	P	2	0	0	0	0	0	2
<i>Alonella exigua</i>	L	2	0	0	0	1	1	0
<i>Rhynchotalona falcata</i>	L	2	1	0	0	1	0	0
<i>Camptocercus rectirostris</i>	L	2	0	1	0	0	1	0
<i>Ceriodaphnia rigaudi</i>	L+P	2	0	0	2	0	0	1
<i>Daphnia curvirostris</i>	P	2	0	0	2	0	0	0
<i>Pleuroxus letourneuxi</i>	L	1	0	0	0	0	0	1
<i>Monospilus dispar</i>	L	1	0	0	0	1	0	0
<i>Picripleuroxus denticulatus</i>	L	1	0	1	0	0	0	0
<i>Alona weltneri</i>	L	1	0	1	0	0	0	0
<i>Alona intermedia</i>	L	1	0	1	0	0	0	0

\*The data are grouped per method (AC method, analysis of pooled active community samples; EB method, incubation of egg bank samples) and per geographic region (DK, Denmark; BNL, Belgium/The Netherlands; and SP, Spain).

†HP is habitat preference according to Flößner (2000): L = littoral, P = pelagic, B = benthic.

‡N is total number of lakes in which the species was detected with at least one of both methods ( $N_{\max} = 88$ ). Species are ranked according to decreasing N.

systems are located in remote areas, rendering the sampling of active communities time consuming and expensive (high transportation costs). Furthermore, egg banks can be sampled at any time of the year. The hatching method may thus facilitate the study of diversity in ephemeral habitats of remote areas.

### Comments and recommendations

In recent years, several studies have assessed the richness of zooplankton taxa through the analysis of egg bank samples. The hatching method has been applied in a Scottish lowland lake (May 1986), in a number of riparian wetlands in the floodplain of the Missouri river (Havel et al. 2000), in a semi-arid reservoir in Brazil (Crispim and Watanabe 2001), in two deep lakes in New Zealand (Duggan et al. 2002), and in shallow European lakes (this study). Most likely, the method can be applied to many other aquatic ecosystems and groups of short-lived organisms. The hatching method may not only be useful to assess richness in zooplankton taxa. With some modifications, it may also successfully be applied for the assessment of diversity in natural communities of bacteria, protists, and plants.

Although promising, the method has also limitations. Because of the constant mixing of sediment layers in aquatic sediments, egg or seed bank analysis cannot be applied to detect short-term changes in species richness. The method also proved unreliable for assessing cladoceran richness in

young pools, since not all populations produce diapausing eggs shortly after establishment (Vandekerkhove et al. 2004c). Another weakness is that diapausing egg production is weak or absent in some species (Jankowski and Straile 2003) or varies with temperature (Jeppesen et al. 2003).

A significant step in the further optimization of the analysis of egg bank samples would include the development of tools that allow the direct species identification of diapausing eggs. Such tools would render the incubation procedure unnecessary and thereby raise the reliability, cost efficiency, and applicability of the analysis of egg bank samples. The bias associated with hatching may be circumvented by compiling keys that allow identification of diapausing stages based on their morphology. Such keys are still lacking for most taxa (but see Vandekerkhove et al. 2004a and Mergeay et al. 2005 for an overview of diapausing eggs of a variety of Anomopod species). An alternative to morphological identification of diapausing eggs may be the application of molecular techniques, similar to the ones used for the characterization of bacterial communities (e.g., DGGE; Muyzer 1999, Van der Gucht et al. 2001). Moreover, the analysis of other identifiable (to species level) remains stored in the sediment, such as carapaces, postabdomens, and headshields (Frey 1986), may be a useful complementary method to the hatching method for the assessment of cladoceran species richness.

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