Krystyna KALINOWSKA¹*, Jolanta EJSMONT-KARABIN¹, Jan I. RYBAK²

¹Centre for Ecological Research, Polish Academy of Sciences, Hydrobiological Station
Lesna 13, 11-730 Mikołajki, Poland
*e-mail: kkalinowska1@poczta.onet.pl (corresponding author)
²Department of Hydrobiology, University of Warsaw, Banacha 2, 02-097 Warsaw, Poland

THE ROLE OF LAKE SHORE SAND DEPOSITS AS BANK OF CILIATE, ROTIFER AND CRUSTACEAN RESTING FORMS: EXPERIMENTAL APPROACH

ABSTRACT: The experiment, with the use of sand deposit from hygroarenal (shore sand beach wetted by lake waves) of the beach of eutrophic Lake Mikołajskie (Masurian Lakeland, Poland), was performed in order to test the hypothesis that shore sand deposit is the bank of resting forms of ciliate, rotifer and crustacean species. The experiment was conducted over a 38 days period in March-April 2004. Frozen sand taken in winter was exposed in aquariums filled with pre-filtered (GF/C) lake water in stable temperature (20°C), oxygen saturation and 12:12 light/dark conditions. Samples (three replicates) were taken each day. A total number of 44 ciliate, 59 rotifer and 9 crustacean taxa were identified during the studied period. Resting forms of different species and/or taxonomic groups developed in different time and it may be the result of different strategies in colonization of new habitats. Organisms forming resting cysts such as ciliates (11 species) and bdelloid rotifers appeared after the first day of the incubation whereas organisms hatching from resting eggs (monogonont rotifers and crustacea) were observed from the 2nd-3rd day of the experiment. The numbers of all the studied groups of organisms increased gradually and then strongly decreased indicating probably nutrient and/or organic matter limitation. The highest numbers of ciliates (731 ind. cm⁻² of sand) was found on the 5th day, rotifers (987 ind. 100 cm⁻²) on the 23rd day, cladocerans (60 ind. 100 cm⁻²) on the 21st day and copepods (30 ind. 100 cm⁻²) on the 33rd day of the experiment. The results of this study suggests that shore sand deposits being the temporal refugium for the small-bodied invertebrates transported here with the wave action may also play an important role as the transfer for their further dispersal in addition to postulated ways of expansions such as wind, rain, animals and surface runoff.

KEY WORDS: psammon, resting forms, ciliates, rotifers, crustaceans

1. INTRODUCTION

Creating the resting forms is the common phenomenon among aquatic organisms. There is relatively large information about the resting stages and life-cycle strategies of rotifers, cladocerans and copepods, whereas ciliate resting forms and their ecological role are poorly understood. It is well documented that resting stages of zooplankton are very abundant and they can survive in dormancy long periods of time (Corliss and Esser 1974, Snell et al. 1983, Fenchel 1987, Hairston 1996). The function of the resting forms is: to survive unfavourable environmental conditions (e.g. food shortage, drying up, evaporation of the surrounding water, changes in temperature and day length), to
avoid the predators as well as to disperse and colonize the new habitats (Corliss and Esser 1974, Hairston 1996, Panov et al. 2004, Schröder 2005).

Rotifers are characterized by the two different strategies that allow them to survive adverse conditions (Ricci 2001). Monogononts produce, after switching to mictic phase, resting eggs (diapause), which are resistant to dry and extreme temperatures. This strategy plays an important ecological role in aquatic ecosystems because allow them to survive long-term unfavourable conditions and to colonize the new habitats. Second type of strategy is typical of bdelloids that enter anhydrobiosis (quiescence) at any time during their life cycle. These two strategies are alternative and mutually exclusive, as no single rotifer species seems capable of both diapause and quiescence. It is commonly believed that rotifers may be spread by the wind, rain and aquatic birds because of resting forms and parthenogenesis. However, results of the studies by Jenkins and Underwood (1998) did not support this opinion. Nevertheless, newly created aquatic reservoirs are very quickly colonized by rotifers (Ejsmont-Karabin 1995).

Resting egg production as the life strategy component of freshwater planktonic cladocerans and copepods is a common life trait. Pelagic copepods possess two types of resting eggs (subitaneous and diapause) that differ markedly in structure and morphology. Subitaneous eggs are produced in normal conditions, they hatch within a few days at ambient temperatures, but they can be induced to become quiescent in response to harsh period, and they have the capacity for short-term survival. Diapause eggs are produced during unfavourable environmental conditions, they do not hatch within a few days even if conditions are favourable, and have the ability to long-term survival (Grice and Marcus 1981, Hairston and Munns 1984, Marcus 1996, Dharani and Altaf 2004). Temperature and photoperiod are identified as the key factors that promote the occurrence of copepod diapause eggs (Chinney and Williams 2003). Other factors responsible for the induction of diapause could be predator avoidance, food depletion, over-population, pH, conductivity, crowding and gradual reduction of water quality or it may be genetically programmed into the phenotype of these organisms for their sustainability in smaller water bodies (Grice and Marcus 1981, Marcus 1996, Dharani and Altaf 2004).

As shown by Weisse (2006) the occurrence of resting stages of ciliates is similar to that of Daphnia. Ciliates form resting cysts when environmental conditions become adverse (Fenchel 1987, Foissner et al. 2005). Among ciliates, cyst formation is well established mainly among oligotrichs and tintinnids which are the characteristic components both of freshwater and marine ciliate community. The resting cysts play an important role in the seasonality and survival strategy (Müller 2000). There is information on factors, which induce encystment or excystment of protozoa, such as changes in pH, ionic composition of the water, temperature, or light (Paranjape 1980, Sako et al. 1984, Müller 2002). The significance, however, of many of these findings is unclear.

The aim of this study was to evaluate whether sandy shore deposit occasionally moisturized by waves (in this case hygroarenal) is functioning as the bank of resting forms of ciliate, rotifer and crustacean species and in this way - the source of organisms able to colonize new habitats.

2. MATERIAL AND METHODS

The experiment was performed over a period of 38 days, from 15 March to 21 April 2004. Frozen sand for the experiment was taken from hygroarenal (shore sand deposits wetted by lake waves) of the beach of eutrophic Lake Mikolajskie (Masurian Land, Poland). Sand was placed in three 70-L aquariums in which it was uniformly distributed. In each aquarium, there was 2 cm layer of sand. Then, aquariums were filled with natural lake water, earlier pre-filtered through 0.45 μm glass filters Whatman GF/C in order to remove all organisms, while leaving the small sized bacterial cells. Water in aquariums was saturated using oxygen pumps. Water temperature was adjusted to 20°C with the immersion heater and maintained at this level throughout the experiment. A 12:12 h light:dark cycle was hold in each aquarium.
Samples were taken every day for 4 weeks and weekly afterwards. Before sampling, sand was mixed in order to enable the light access to organisms living in deeper part of sand, and water samples responding the sand area of 20 cm² were taken. The samples (three replicates, volume about 500 mL) were filtered through 10 μm (for ciliates) and 30 μm mesh net (for rotifers and crustaceans) and fixed with Lugol’s solution and then in the case of rotifers and crustaceans with 4% formalin. Numbers of organisms were calculated per square centimeter of sand.

3. RESULTS

3.1. Ciliata

Ciliates appeared already after one day of the incubation. A total of 44 species were found throughout the experiment. Most of them (11 taxa; 25% of the total number of species) were present immediately after change of environmental conditions on favourable for growth and reproduction. All recorded taxa were detected within 11 days of the experiment. It is characteristic that the first ciliates, which restarted metabolic activity, were almost exclusively small forms. Among them bacterivorous Aspidisca spp. (benthic) and Cyclidium heptatrichum Schewiakoff (benthic and planktonic), typical planktonic algivorous Codonella cratera (Leidy), Rimostrombidium humile (Penard) and Rimostrombidium lacustris (Foissner et al.) as well as planktonic/benthic species feeding on both bacteria and algae (Urotricha spp.) were observed. Large, mainly benthic forms (e.g. Frontonia sp., Loxocephallus luridus Eberhard, Spirostomum minus Roux, Stylochlamys mytilus-complex, Uroleptus sp.) appeared later.

The pattern of appearance of particular ciliate orders is somewhat similar (Fig. 1). After distinct increases at the beginning of the experiment, ciliate densities decreased and then remained at almost constant and low level until the end of the experiment. Cyrtophorida were the first to reach maximal abundance, after which their density dramatically decreased to very low level of several individuals per 1 cm². The marked increase of small planktonic forms belonging to the orders of Oligotrichida, Scuticociliatida and Prostomatida was observed. However, their maximal abundances were two or three times higher than the numbers of Cyrtophorida. Other ciliates, including orders of Haptorida, Hymenostomatida, Hypotrichida and Peritrichida, showed different patterns. However, they fluctuated on relatively low level during the whole time of the experiment and did not show distinct increases and drastic decreases.

During the first 5 days of the experiment, the total ciliate numbers increased rapidly.

![Fig. 1. Changes in densities of the major ciliate orders during the experiment (mean values from three replicates).](image)
from 59 ± 9 to 731 ± 138 ind. cm⁻² of sand (Fig. 2). During the following days, the numbers sharply decreased to about 100 ind. cm⁻² and remained on this level with small fluctuations until the end of the experiment.

3.2. Rotifera

In total, 58 species of rotifers were found during the experiment. Similar to ciliates, rotifers appeared on the first day of the incubation. These were, however, exclusively species belonging to the order of Bdelloidae, that were able to survive adverse conditions, such as low temperature and food depletion, in dormant stages (anhydrobiosis). On the second day of the experiment, members of monogononts represented by two benthic species *Cephalodella gibba* (Ehrenberg) and *Colurella hindenburgi* (Steinecke) appeared. On the 3rd day, eleven species were present in samples, among which mainly the most common planktonic *Keratella cochlearis* (Gosse) and *Polyarthra dolichoptera* (Idelson) as well

![Fig. 2. Changes in total ciliate numbers during the experiment (mean from three replicates ± SD).](image)

![Fig. 3. Changes in numbers of dominating genus of rotifers during the experiment (mean values from three replicates).](image)
Lake shore sand as bank of resting forms as typical psammic forms such as *Lecane clara* (Bryce), *L. levistyla* (Olofsson), *L. psammophila* (Wiszniewski) occurred.

Bdelloids were numerous from the first days of the experiment. Their numbers, after initial 3-fold increase, did not change drastically during the following days of the study. Members of these rotifers were present throughout the study period (Fig. 3). In contrast, species belonging to Monogononta that hatched from resting eggs appeared after several days of incubation. In this case, only *Lecane* reached higher densities than bdelloids. This genus was strongly dominated by eurytopic, very common both in periphyton and psammon communities – *Lecane closterocerca* (Schmarda) that reached maximal abundance on the 18th day of the experiment and then, after several days, decreased markedly. The numbers of other species remained at relatively low level during the whole time of the experiment.

The total rotifer numbers increased gradually reaching maximal value of 987 ± 452 ind. 100 cm⁻² on the 23rd day of the exposition and then decreased to values of about 4-times lower (Fig. 4). The most abundant species *Lecane clasterocerca* decided on the general character of variations in the total numbers.

3.3. Crustacea

A total number of 9 crustacean taxa were identified. Among them, three cyclopoid copepods *Eucyclops macrurus* (Sars), *Diacyclops bicuspidatus* (Claus) and *Mesocyclops leuckarti* (Claus) were found. After three days of the experiment five individuals of the pelagic cladocerans *Daphnia hyalina* Leydig, hatched from ephippium. During the next days of the incubation subsequent individuals of this species appeared, among which some, already after five days, had eggs in brood chamber indicating their parthenogenetic reproduction (Fig. 5A). *Ceriodaphnia quadrangularis* (O.F. Müller) and, the most common in littoral zone, *Disparalona rostrata* (Koch) hatched on the 9th day of the experiment. These both cladoceran species, similarly as *D. hyalina*, had embryos in brood chambers. It may suggest that cladocerans had met good food conditions in this experiment. From the 16th day of the incubation, cyclopoid adult individuals appeared. Possibly the first were juvenile copepods and then, after 16 days of the experiment, adult forms of the species common in lake littoral occurred (Fig. 5B).

The total number of cladocerans fluctuated considerably during the whole experiment.
Fig. 5. Changes in numbers of cladocerans (A) and cyclopoid copepods (B).

4. DISCUSSION

The results of this experiment showed that sandy littoral (sand from hygroarenal) might be a source of many species that are able to colonize new habitats or habitats that are periodically devoid of organisms in the consequence of dry and freezing conditions. It is interesting that not only littoral and psammon forms may hatch from sand but also species that are typical of pelagial. Planktonic organisms were represented by 5 ciliate (Codonella cratera, Rimostrombidium lacustris, Strombidium, Coleps spatia Foissner, Paradileptus elephasius (Svec), 10 rotifer (Keratella cochlearis, K. quadrata (Müller), Polyarthra dolichoptera, Filinia longiseta (Ehrenberg), Pompolyx sulcata Hudson, Synchaeta pectinata Ehrenberg, S. kitina Rousselet, Anturaeopsis fissa (Gosse), Trichocerca similis (Wierzejski), Brachionus angularis Gosse) and 3 crustacean species (Daphnia hyalina, Daphnia cucullata Sars, Diacyclops bicuspidatus (Claus)).
All the three studied groups of organisms followed almost a similar trend. They increased sharply and after reaching maximum decreased rapidly towards the end of the incubation (Fig. 6). However, their maxima were noted at different times. The highest number of ciliates was found during the first days (4–5 d) of the experiment. Rotifers were the most numerous in the middle (20–23 d) whereas crustaceans at the end of the experiment (30–33 d). Weiss (1990) found that in the short-term studies, grazing pressure by the higher trophic levels and substrate availability are the two major control mechanisms regulating densities of organisms. According to Pennak (1951) organisms living in sand are dependent mainly on organic detritus as a basic food source as well as on algae and bacteria. In our experiment species that might be responsible for ciliate reduction were filter-feeding cladocerans *Daphnia* spp. and *Ceriodaphnia*, copepods as well as several filter-feeding rotifers. However, as shown by Wickham *et al.* (2000) meiofauna, mainly ostracods have impact on benthic ciliates and reduce their final abundance. We did not observe these large organisms in our experimental aquariums. Thus, it appears that a lack of large predators and limited food resources during the first days of the experiment might directly cause high increase in the total numbers of the studied groups of organisms. The low abundances of these organisms during the second half of the experiment might be attributed to exhaustion of nutrients and organic matter. Thus, availability of food (mostly detritus) and nutrient concentrations in lake water seems to be the main factor influencing abundance as well as, to some extent, structure of psammon communities.

In our experiment, a remarkably rapid appearance of species was observed. Ciliates and bdelloid rotifers were the earliest to appear (first day of the incubation). These two groups of organisms can able to survive unfavourable conditions in dormant stage. Our results are in accordance with these of Laybourn-Parry (1992) who reported that the whole excystment process of protozoa appears to span up to 24 h. Müller (2002) also showed that active strombidid ciliates appeared after 1 d of incubation under warm/light conditions. After several days, forms hatching from resting eggs such as monogonont rotifers and crustaceans appeared in our experiment. It seems that in the case of resting eggs two strategies may be realized. First strategy is characteristic of common and often reaching high abundance during the first days of the experiment might directly cause high increase in the total numbers of the studied groups of organisms. The low abundances of these organisms during the second half of the experiment might be attributed to exhaustion of nutrients and organic matter. Thus, availability of food (mostly detritus) and nutrient concentrations in lake water seems to be the main factor influencing abundance as well as, to some extent, structure of psammon communities.

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ubiquitous forms such as, for example, *Lecane clusiotericera*. It is opportunistic ("wasteful") strategy that based on the production of large numbers of resting eggs from which many forms hatch when favourable conditions immediately return. Moreover, light and temperature are the factors that trigger this response. Second strategy called specialist ("cautious"), is realized by less abundant and more specialized species producing resting eggs rarely and less numerous. Probably these animals hatch lately, when favourable environmental conditions are stabilized.

Results of this study are consistent with others studies. For example, Bailey et al. (2003) indicated that rotifers *Asplanchna girardi* (De Guerne), *Brachionus budapestinensis* Daday, and *B. calyciflorus* Pallas generally began to hatch within 24 h of incubation, while calanoid copepods and cladocerans *Bosmina* *liederi* De Melo and Hebert and *Daphnia longiremis* G.O. Sars began to hatch at 3rd day. Bartholmë et al. (2005) reported that about 70–80% of the calanoid eggs and 35–50% of the cyclopoid eggs released nauplii within 3 days. Similar conclusion was formulated by Madhupratap et al. (1996) who found that resting eggs of four species of calanoid copepods hatched from sediments up to 5 cm depth within 24 to 48 h of incubation.

In the published sources, there are many reports on agents, which may induce encystment or excystment of protozoa, such as changes in pH, oxygen and ionic concentrations in the water, temperature and light. Paranjape (1980) and Šako et al. (1984) reported that besides these various factors inducing fast (shorter than 24 h) excystment, especially grazers’ presence/absence and the availability of nutrients and organic matter may play the most important role in development of protistan cysts, especially ciliates. According to Corliss and Esser (1974) and Hamels et al. (2004) many forms of ciliates excyst when food availability, mainly bacteria and microalgae, improves. As shown by Weisse (2006) and Laybourn-Parry (1992) temperature, in addition to food, had the strongest impact on the ciliate population growth rates. Experiment with cysts conducted by Müller (2002) revealed that cyst age and temperature are the main factors controlling excystment of *Pelagostrombidium* sp. According to the author, comparison of the results obtained under warm/light and warm/dark conditions does not give a clear picture of the impact of light on excystment. Other factors responsible for ciliate excystment, especially tintinnids from marine sediments, are the abundance of phytoplankton species and their extracellular products (Kamiyama 1994, 1997). As shown by Radwan (1984) the main factor, which determines the occurrence and abundance of rotifers, is temperature. May (1987) underlined, that the effect of incubation temperature on the hatching of rotifer resting eggs in sediment samples varied among the species studied. The conditions required to initiate development of resting eggs of *Daphnia* species are also species-specific, although the ambient temperature and light are shown to affect their hatch success (Schwartz and Hebert 1987). Moore et al. (1996) underlined that temperature close to or slightly higher than 20°C are optimal for cladocerans. In our experiment the temperature was 20°C and it seems that it was optimal for the growth of the studied organisms. These results show that not only, as was mentioned above, food availability, but also favourable environmental conditions such as temperature and light, have an important impact on the ciliate as well as rotifer and crustacean development.

Published data show that resting stages play a significant role in dispersal of aquatic invertebrates and in colonization of new habitats (Panov et al. 2004). As given in Havel and Shurin (2004) there are different mechanisms and vectors of dispersal, among which transport by wind, flowing water, animals and human-mediated effects are probably the most important. The authors suggested that zooplankton dispersal processes may regulate biodiversity, species composition, and genetic structure in reservoirs, lakes, and ponds. Experimental studies conducted by Bailey et al. (2003) demonstrate that natural or anthropogenic movement of sediments may be a crucial vector for the dispersal of invertebrate resting stages between water bodies. Results of these investigations using ships’ ballast sediments indicated that rotifers and copepod nauplii hatched from ballast sediments and that the dispersal of diapausing eggs contained in residual sediment may be a crucial invasion.
mechanism. However, Bailey et al. (2005) have recently shown that hatching of diapausing eggs contained in ballast sediment of ships poses a relatively low risk of invasion to the North American Great Lakes. Based on the experimental results of this study, we suggest that sand shore deposits may play an important role in the dispersal of small aquatic invertebrates, such as ciliates, rotifers and crustaceans. Most protozoa have a high potential for dispersal and their cysts, which resist desiccation, can be spread by the wind over great distance, but even species, which do not form such cysts can be transported over long distances on or in aquatic birds and insects, or on the feet of mammals (Reville et al. 1967). Taylor (1981) found that many aquatic species form cysts in response to exhaustion of their food supply, rather, than searching for a new area. As shown by Schmid-Araya (1998) rotifers reproduce very quickly and can rapidly occupy vacant niches opportunistically.

In conclusion, sand deposits in the littoral zone of lakes occasionally affected by wave action constitutes a bank of resting forms of many small-bodied species of different groups of organisms such as ciliates, rotifers and crustaceans. The occurrence of resting stages in this special habitat is unpredictable and dependent on the frequency of spray by lake waves in the season preceded by resting period. Thus, taxonomic composition and densities of organisms are accidental because species derive from pelagial, damaged periphyton as well as are raised from the bottom. However, despite of this accidental occurrence and retaining of resting forms their further development in the conditions of contact with lake water suggests that sand deposits in this littoral site may play an important role in the dispersal of small-bodied invertebrates in addition to postulated ways of expansions such as wind, rain, animals and surface runoff. Resting forms of different species and/or taxonomic groups after releasing develop in different time and it may be included in their life strategies and in colonization of new habitats. The numbers of all the studied groups of organisms initially increased and then strongly decreased indicating that the pool of nutrients and in consequence limited amounts of available food in the experimental conditions did not allow reach as high numbers of organisms hatching from sand as in sandy shore in natural conditions (Ejsmont-Karabin 2003, Kalinowska 2008).

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5. REFERENCES


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