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Restoration of tropical peat swamp rotifer communities after perturbation: an experimental study of recovery of rotifers from the resting egg bank

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Abstract

In order to assess the recovery potential of tropical freshwater communities after disturbance, we performed an experimental study on the effects of exposure conditions and durations of storage on hatching of rotifer resting eggs in sediment. Well-mixed surface sediment samples from Mai Khao peat swamp on Phuket Island, Thailand, were stored under three conditions (cold –4 °C & dark: CD; ambient –32–42 °C & dark: AD; and ambient & daylight conditions: AL), for different periods of time (1, 2, 4, 6, 12, 18 and 24 months).The number of species hatching from the sediment was significantly affected by treatment for both short- (1–6 months) and long-term (6–20 months) exposure. Significant effects of short- and long-term exposure within treatments were also present. Both factors interacted significantly. Regarding numbers of specimens hatching, no short-term effects of differences in treatment condition were found, but increasing the duration did have an effect. Significant effects of treatment occurred after 6 months, in addition to prolonged effects of duration. Again, both factors interacted significantly. These experiments indicate that exposure time has a strong impact on the viability of resting eggs, whereas, an effect of exposure condition appears only after 6 months. So, recovery of rotifer communities from resident sediment egg banks in disturbed peat swamps can only be effectively attained when restoration occurs within a relatively short period after perturbation.

Introduction

Presently, the most severe threat to the world's wetlands is posed by land uses that destroy or severely damage habitats (Finlayson & Moser, 1991). Human-induced pressures affect ecosystem functioning as well as biodiversity at all levels, from ecosystems to organisms, Whereas populations of some organisms are irreversibly affected, others may be able to recover from the effects of disturbances. This resilience results at least partly from their potential to survive periods of adverse conditions through resistant, dormant stages.

Monogonont Rotifera, being of prime ecological importance in freshwater ecosystems, has resting eggs or cysts as dormant stages (Gilbert, 1974). These are diapausing embryos produced by fertilized mictic females. Sexual reproduction is induced by a variety of cues including the occurrence of environmental changes associated with habitat deterioration. Hatching of these resting eggs generally occurs in coincidence of favorable conditions in the habitat, and results in the re-establishment of populations (Pourriot & Snell, 1983; Ricci, 2001). Resting eggs thus represent a biodiversity bank, as they can assure genetic continuity through periods of hazardous environmental conditions and offer a recolonization resource when favorable conditions return (Pourriot & Snell, 1983; Ricci, 2001).

So far, the majority of studies on rotifer resting eggs consist of investigations on resting egg production and hatching, often in relation to the use of rotifers as food source in aquaculture (Lubzens et a1., 1980, 1993; Pourriot et al., 1980; Minkoff et al., 1983; Serrano et al., 1989). There are few studies on resting eggs in natural rotifer populations. Ito (1958) and Nipkow (1961) were amongst the first to study incubation of rotifer resting eggs from sediments (May, 1987). Pourriot et al. (1984) and Gilbert & Wurdak (1978) compared the morphology of resting eggs of different taxa. May (1987) performed a quantitative study of rotifers hatching from sediments from Loch Leven, Scotland, and recorded species-specific effects of temperature on the emergence of rotifers, and showed that all pelagic rotifer species found in the lake could be hatched from the sediment egg bank. Langley et al. (2001) investigated the relative importance of recruitment from the resting egg bank vs. passive dispersal in the recolonization of temporary ponds, and found that the former is by far the most important source. These studies clearly show the potential importance of resting egg banks in the restoration of rotifer communities after disturbance. However, there are several hiatuses remaining (see Ricci, 2001). For instance, no information is available on rotifer resting egg banks in tropical habitats, and little is known on any but pelagic rotifer taxa.

As for the diversity of tropical habitats, one of the most intriguing habitats is that of peat swamp forest. Previous studies on the diversity of monogonont Rotifera in peat swamps suggest that this ecosystem type has a diverse rotifer fauna, as a result of its long history and unique ecological characteristics (Chittapun et al., 1999, 2002, 2003; Chittapun & Pholpunthin, 2001; Segers & Chittapun, 2001). Unfortunately, these habitats are seriously threatened by human activities such as agriculture (e.g., transformation to arable land, eutrophication) and aquaculture (e.g., salinization resulting from discharge of saltwater from shrimp farms). These activities constitute serious threats to the general biodiversity, and diversity of Rotifera Monogononta in particular, of these ecosystems in Thailand. In order to assess if, and to what extent rotifer communities can recover after restoration of these peat swamps, we studied the recruitment of rotifers from the sediment resting egg bank stored under different condition and duration of exposition.

Materials and methods

Study area

Mai-Khao is one of the six remaining peat swamps located along Mai-Khao coast on Phuket Island, Southern Thailand (Fig. 1). Historically, the different peat swamps in the area were connected, but they are now isolated and diversified ecologically due to different human activities in each fragment. Mai-Khao peat swamp has recently become brackish as it received discharged saltwater from nearby aquaculture farming. As a result, the once thriving macrophyte vegetation has disappeared, and the accumulated layer of peat is decomposing. Because of the decline of macrophytes, the sediment in its shallow areas is now exposed to direct sunlight during the dry season.

Sediment collection, treatment and incubation

Sediment including resting eggs was collected randomly from a dry area of Mai-Khao peat swamp on 27 February 2000, yielding a total of approximately 5 kg of material. To avoid excessive differences among resting egg ages, only the top 1 cm of sediment was scraped off from the soil. The sediment was allowed to dry further under a paper cover for a month. Then, it was homogenized by removing large pieces of plant material, grinding and passing it through a 0.5 cm mesh sieve. The sediment was then divided in three equal parts, which were subjected to different treatments:

- Cold-Dark (CD): Sediment stored in an opaque box, and kept in a refrigerator (24 °C). This condition was assumed to reflect the optimal condition to retain viability in the resting eggs;
- Ambient-Dark (AD): Sediment stored in an opaque box, under ambient temperature. Condition reflects that of resting eggs deep in the sediment;

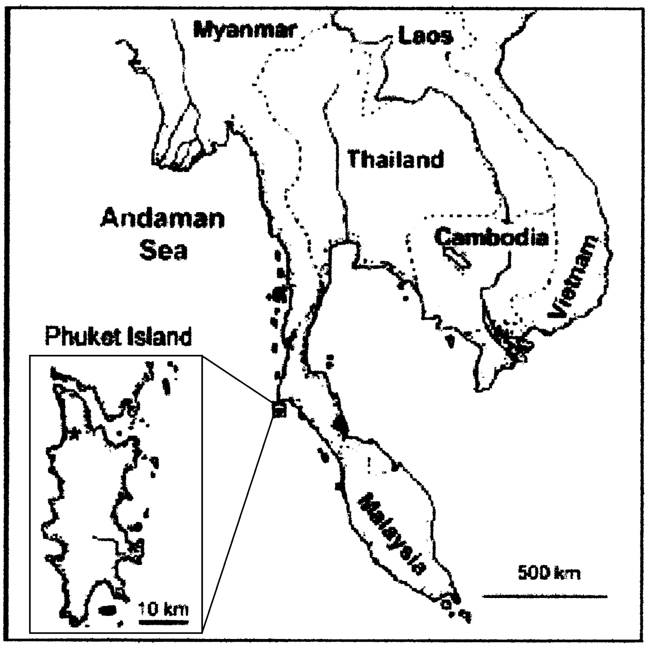

Figure 1. Location of Mai-Khao peat swamp (*) on Phuket Island, Southern Thailand.

- Ambient-Light (AL): Sediment stored in a translucent box, under ambient temperature. Condition reflects that of completely exposed resting eggs.

The sediment boxes in the 'ambient' treatments were placed together in a water bath, in order to keep the temperature in the boxes similar, and placed under a clear roof in the culture laboratory, Temperature varied in this treatment, ranging from 27 to 42 °C, but remained similar in both boxes. The duration of the treatments varied from 0 months (initial experiment), to 1, 2, 4, 6, 12, 18 and 24 months (Fig. 2).

Hatching of rotifer resting eggs was tested by placing exactly 20 g of sediment into 250 ml beakers, and adding 150 ml of distilled water. Each test was replicated four times. The beakers were placed in an incubator (Jermaks) at 28 °C, with a 12 h light–12 h dark light regime. Every four days during 3 months, the water in these beakers was poured out into a different vial, and topped back to the same level in the original

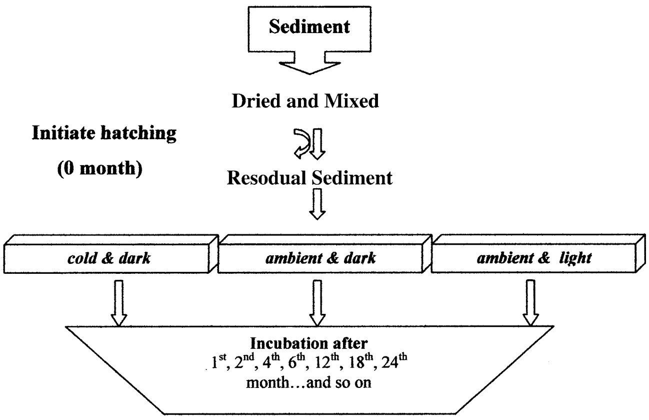

Figure 2. Flow-chart of the experiment.

beaker. To collect and count the rotifers hatched during each 4-days interval, formalin was added to the collected water to a final concentration of about 5%. Rotifers were then sorted and counted under an Olympus VM dissecting microscope, and identified using an Olympus CH-2 compound microscope. As few individuals, and no additional species were found to hatch after 3 months in an initial test experiment, the observations were stopped after this period.

Data analysis

Data on emerging of rotifers after different exposure conditions and durations were analyzed by applying Repeated Measures Analysis (SPSS statistical package for Window, Release 11.0.1). Analyses were performed on two different aspects of data: number of species emerging, and number of individual specimens hatching. To examine the combined effect of exposure condition and duration on the diversity of the rotifers hatching, each group of data was separated into two time periods. First, we tested the data for a short-term effect, by analyzing results from 2 months intervals over a period of 6 months (0, 2, 4 and 6 months); second, we tested for long-term effects by analyzing results from six months intervals over a total period of 24 months (0, 6, 12, 18 and 24 months).

Results

We analyzed the effect of treatment and exposure time on rotifer hatching by considering two aspects of diversity, viz. number of species and number of individuals.

Number of species

The number of species hatching from the sediment was affected significantly by exposure conditions, both in the short- and the long term (F = 4.97 and 10.37, $p < 0.05$, df = 2). Significant effects of short- and long-term exposure within treatments were also present (F = 20.94 and 66.25, $p < 0.01$, df = 3 and 4, respectively). Both factors interacted significantly (short-term: $F = 4.60$, $p < 0.01$, df = 6; long-term: $F = 2.68$, $p < 0.01$, df = 8) (Table 1).

The results demonstrate that rotifer species diversity was affected by exposure conditions. The highest number of species hatched from sediment kept under cold and dark conditions, fewer hatched from sediments kept in ambient temperature and in the dark, and the lowest number was recorded from sediment kept in ambient and light conditions (Table 2). This effect is significant even after short-term storage, but is especially obvious when comparing long-term effects (Fig. 3). After 24 months of storage, hatching of rotifers could only be observed from sediments stored under cold and dark conditions.

The number of individuals hatching

The number of rotifers hatching initially from the sediment amounts to 470–956 per gram. No short-term effects of differences in treatment conditions on the numbers of rotifers hatching were found ($F = 0.68$, $p > 0.05$, df = 2), although an

Table 1. Repeated measurement analysis of the short- and long-term effect of exposure condition and duration on the number of species hatching

Source	Type III Sum of squares	df	Mean square	F	Sig.
Short-term effect					
Duration	26.896	3	8.965	20.935	0.000
Exposure condition	2.260	2	1.130	4.969	0.035
Duration*Exposure condition	11.792	6	1.965	4.598	0.002
Long-term effect					
Duration	206.100	4	71.304	66.246	0.000
Exposure condition	3.227	2	1.613	10.371	0.005
Duration*Exposure condition	16.700	8	1.965	2.684	0.020

Table 2. Rotifer species hatching from the sediment exposed to different conditions

Species	Start	CD	AD	AL
Brachionus rotundiformis		+		
B. urceolaris	+	+	+	+
Cephalodella gibba	+	+		
C. innesi		+		
Encentrum pornsilpi		+	+	
Floscularia conifera	+	+	+	
Hexartha mira				+
Lecane bifurca		+	+	+
L. bulla	+	+	+	+
L. inermis		+	+	+
L. ludwigii		+	+	
L. obtusa	+	+	+	+
L. tenuiseta	+	+	+	+
L. unguitata	+			
Lindia torulosa		+		
Trichocerca pusilla		+		
T. tenuior		+		
	7	15	9	7

increase in duration did have an effect ($F = 6.55$, $p < 0.01$, df $= 3$). Significant effects of treatment occurred after 6 months ($F = 14.83$, $p < 0.01$, df $= 2$), in addition to prolonged effects of duration ($F = 42.00$, $p < 0.01$, df $= 4$). Again, both factors interacted significantly (short-term: $F = 0.54$, $p < 0.01$, df $= 6$; long-term: $F = 9.05$, $p < 0.01$, df $= 8$) (Table 3).

The results point out that time also has a significant effect on rotifer diversity in terms of number of specimens hatching. An additional effect of exposure condition only becomes significant after 6 months. As before, cold and dark conditions appears to affect hatching the least (Fig. 4).

Discussion

Species composition

Throughout the 2 years of the experiment, 17 rotifer species emerged from the sediment (Table 1). This equals to only 23.5% of the total rotifer record from the swamp. One species, *Lindia torulosa*, emerged from the sediment but was never found in regular plankton samples collected in the swamp. This discrepancy is not unexpected. Evidently, it reflects the difference in sampling intensity between the zooplankton survey (ca. 10 vertical hauls in different parts of the swamp monthly, over a period of 16 months) and the collection of sediment for the experiment (point sample). Moreover, it is unlikely that the single sediment sample adequately reflects the habitat heterogeneity of a shallow peat swamp in the composition of its resting egg bank. It should also be noted that the majority of species recorded in the zooplankton samples are littoral or benthic animals, and it is known that at least some of these attach their resting eggs to a substratum, or are otherwise selective in this respect. Hence, some rotifers inhabiting Mai-Khao peat swamp may not have been present as resting eggs in the sediment collected for the experiment. Additionally, as we collected exposed sediment, it cannot be excluded that particularly vulnerable taxa may already have been eliminated from the active resting egg bank. Finally, the incubation procedure applied in the experiment may not have generated the necessary cue for hatching of some taxa.

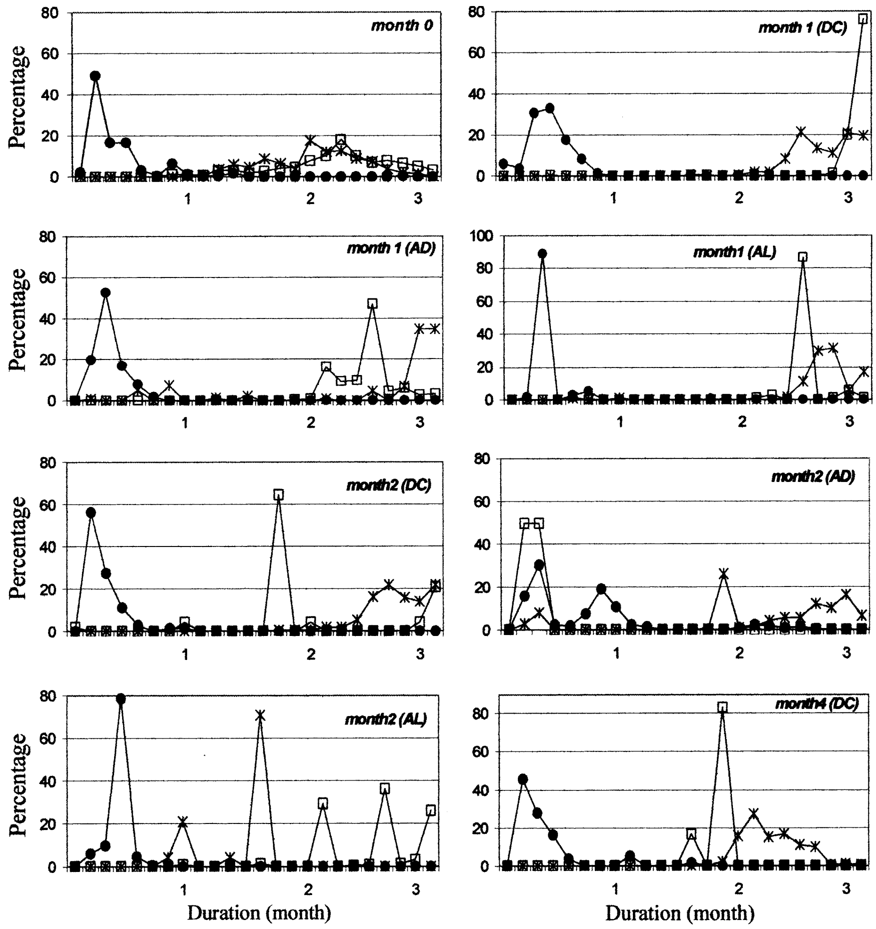

Figure 3. The number of species hatching from different exposure conditions and durations (mean ± 1SE.) (□ = cold and dark, □ = ambient and dark and ▲ = ambient and light).

Table 3. Repeated measurement analysis of the short- and long-term effect of exposure conditions and duration on the number of rotifers hatching

Source	Type III sum of squares	df	Mean square	*F*	Sig.
Short-term effect					
Duration	48.474	3	16.158	6.546	0.002
Exposure condition	0.267	2	0.134	0.681	0.530
Duration*Exposure condition	7.971	6	1.329	0.538	0.774
Long-term effect					
Duration	515.447	4	128.862	42.004	0.000
Exposure condition	25.949	2	12.974	14.826	0.001
Duration*Exposure condition	148.523	8	18.565	9.052	0.000

A striking observation is that the first species to emerge from the sediment invariably turned out to be *B. urceolaris* (Fig. 5). More than 50% of *B. urceolaris* individuals hatched within two weeks of incubation. Both observations support the hypothesis that *B. urceolaris* is a pioneer species, and suggest that the species responds relatively quickly to environmental cues.

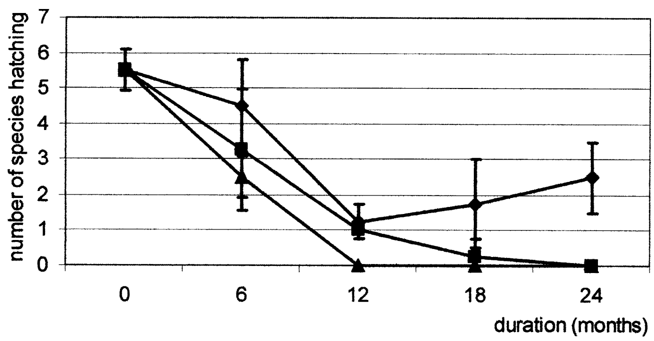

Figure 4. The number of specimens hatching of all species and of the three commonest species from different exposure conditions and durations (mean ± 1SE.): 5a = all species, 5b = *B. urceolaris,* 5c = *L. bulla,* 5d = *L. obtusa* (□ = cold and dark, □ = ambient and dark and ▲ = ambient and light).

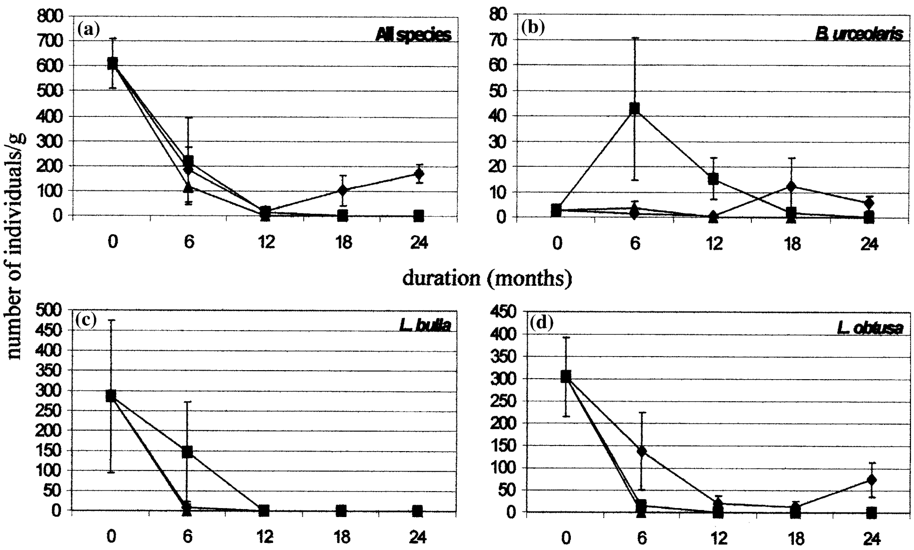

Figure 5. Percentage of hatching of the three commonest species for 3 months (● = *Brachionus urceolaris*, □ = *Lecane bulla*, * = *L. obtusa)*.

Effects of treatment and exposure time

Exposure time plays an important role in the recovery of rotifer diversity from the sediment egg bank. The longer the sediment egg bank is stored, the lower the number of species and individuals of rotifer that emerge. Our observations clearly demonstrate that resting eggs have a limited, and probably species-specific viability. The results we obtained for various rotifer species are in contrast with the report on *Brachionus plicatilis*-group, in which 100% of resting eggs desiccated for up to 6 months can be made to hatch (Lubzens et al., 1980). This variability in resting egg duration is further illustrated by Kotani et al., (2001), who report hatching of resting eggs of *B. rotundiformis* of over 60 years old. In addition, we here present the first quantitative data indicating that the time lapse between dehydration and effective hatching also varies between species.

Exposure conditions have obvious effects after 6 months of storage. There is a significant difference in the number of species and individuals hatching after exposure to cold and dark conditions, in comparison to resting eggs exposed to ambient temperatures and light conditions. That cool and dark conditions extend diapause, and increase the viability of stored rotifer resting eggs, has been reported by many researchers (Pourriot et a1., 1980; Minkoff et al., 1983; Pourriot & Snell, 1983; Hagiwara & Hino, 1989). The lower temperature and absence of light may prevent degradation of compounds, and/or inhibit bacterial development damaging the resting eggs.

Conclusions

Our results demonstrate a strong effect of duration on diversity both in terms of species richness and in number of specimens hatching. Exposure conditions start having significant effects after periods as short as 6 months. This contrasts with general views that rotifer resting eggs are effective for long-term survival of rotifers (e.g., Nogrady et al., 1993). It should be borne in mind that most studies on rotifer diapause are conducted on material stored under optimal conditions (cold and dark), which may not realistically reflect natural conditions, especially when dealing with tropical organisms. This may result in over-estimating the significance of resting egg banks as source for re-establishing populations in nature. The results presented here show that rotifer resting eggs have only a limited viability, and may not be effective in serving as source for recovery of rotifer diversity, even for short-term disturbances.

So, recovery of rotifer communities from sediment egg banks in disturbed peat swamps can only be effectively attained when restoration occurs within a relatively short period after perturbation.

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