Available online at www.sciencedirect.com
ScienceDirect
European Journal of Protistology 43 (2007) 87–94
European Journal of PROTISTOLOGY
www.elsevier.de/ejop

Cryptic freshwater amoeba species in the bottom sediments of Nivå Bay (Øresund, Baltic Sea)

Alexey V. Smirnov*

Marine Biological Laboratory, University of Copenhagen, Helsingør, Denmark and Department of Invertebrate Zoology, St. Petersburg State University, Russia

Received 5 December 2005; received in revised form 26 October 2006; accepted 16 November 2006

Abstract

An aquarium containing a portion of freshly collected brackish-water sediment from Nivå Bay (Øresund, Baltic Sea) was sampled to determine the species of amoebae present and refilled with inorganic salt medium to achieve freshwater salinity. After 2 months incubation, the diversity of amoebae was again studied, and the salinity was restored to the original level. The aquarium was incubated for a further 2 months and the amoeba fauna was studied for the last time. A number of freshwater species appeared in the sample after the first salinity shift, while some marine species disappeared. Most marine species did not re-appear after the salinity in the aquarium was restored, but one marine species not noted previously was recorded. The experiment illustrates the presence of 'cryptic diversity' of amoebae in natural habitats and demonstrates that laboratory manipulation of the salinity of a sample prior to inoculation may achieve an increased recovery of species from a brackish-water habitat.

Keywords: Amoeba; Ecology; Biodiversity; Salinity tolerance; Cryptic diversity

Introduction

Many species of marine amoebae can tolerate a wide range of salinity and even freshwater conditions (Hauer et al. 2001; Hülsmann and Galil 2001; Page 1974, 1983; Sawyer 1975a, b; Smirnov 2001a, b). However, nominally marine amoeba species were never isolated from freshwater. By contrast, nominally freshwater amoeba species that cannot tolerate much increase in salinity in experiments were isolated from low-saline habitats, such as shallow coastal waters of the Baltic Sea (Garstecki and Arndt 2000; Schmöller 1961, 1964; Vørs 1992) and even full-strength sea water of other seas and oceans (Davis 1978; Davis et al. 1978; Sawyer 1980; Sawyer and Bodammer 1983; Sawyer et al. 1993, 1997).

At first sight, these findings raise doubts concerning the statement of Page that long experience has failed to reveal any gymnamoeba occurring both in fresh and salt water (Page 1988). However, the use of enrichment cultures to recover species may result in the isolation of species that do not live and multiply in the habitat, being unable to find appropriate conditions, but persist once they are introduced there, e.g., by water and air currents (Butler and Rogerson 2000; Esteban and Finlay 2003; Fenchel and Finlay 1995; Jorissen et al. 1998; Moodley et al. 1997; Smirnov 1999, 2002). The finding of amoebae at inappropriate salinities may well be another reflection of this phenomenon. Fenchel et al. (1997) used the term 'cryptic diversity' to recognize a pool of rare or

*Correspondence address. Department of Invertebrate Zoology, Faculty of Biology and Soil Sciences, St. Petersburg State University, Universitetskaja nab. 7/9, 199034 St. Petersburg, Russian Federation.
E-mail address: polychaos@mail.ru.

0932-4739/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ejop.2006.11.002

dormant species that are actually present in a habitat but are not revealed during routine faunistic survey. These species cannot find appropriate niches at the moment and have to persist until the conditions change in their favour (Finlay et al. 1997). A shift of conditions is required to provide niches for these species to multiply above the detection limit. Hence, the potential presence of a 'cryptic' fraction of species diversity must be taken into account when designing experiments that involve recovery of microbial species.

Smirnov (2003) showed that the choice of enrichment media used for recovery of amoebae from a freshly collected freshwater sample is important for faunistic analysis; a properly chosen set of media considerably increases the number of species recovered. In marine samples there is an extra variable of critical importance – the salinity. The present paper reports on an experimental attempt to increase the number of amoeba species recovered from a brackish-water habitat by manipulating the salinity in an artificial mesocosm prior to inoculation of samples into enrichment media. This technique allowed recovery of a range of freshwater and marine species which were not detected in the initial sample.

Materials and methods

On February 3, 2004, a 3-litre sample from the top 10 cm layer of sediment in Nivå Bay (Øresund, Baltic Sea, 15 km South from Helsingør, Denmark) was collected with a wide plastic dish and placed on site in a sterile, covered plastic aquarium, 22 cm × 30 cm × 10 cm in size (Figs 1 and 2). The sampled habitat is a brackish-water, coastal bay, two hectares in extent, permanently covered with Øresund water and thus nominally a marine habitat (see Fenchel 1969); the water depth at the site of sampling was about 30 cm. The salinity in the bay at the moment of sampling was 17‰; normally it ranges between 10% and 24‰. On arrival at the laboratory, the aquarium was sampled for the first time, and was thereafter continuously aerated. The sampling procedure was always the same, as follows: 20 mini-cores about 1 ml each were collected from the aquarium so that sampled sites formed a regular grid covering the entire area of the aquarium; the side samples were located near the walls of the aquarium to include species that may require light for their development. Mini-cores were taken with a cut plastic pipette penetrating the entire layer of sediment and gently but carefully mixed together in a glass beaker (Fig. 3). Material from the beaker was inoculated into six sets of dishes. Each set comprised ten 90 mm and ten 60 mm Petri dishes (from my experience, some amoeba species preferably develop in dishes of only one of these sizes). The amount of material inoculated into each dish was about 0.05 ml. Each set of dishes was filled with artificial sea water of a different salinity (5‰, 15‰, 30‰, 50‰ and 70‰); one set was filled with freshwater PJ (Prescott and James 1955) medium. The salinities exceeding that in the initial habitat were used to check the overall range of salinity tolerance. Each medium contained 0.01% cerophyl infusion (Page 1988) as a source of nutrients. All media were autoclaved prior to use. Dishes were incubated under room conditions and examined twice with an inverted microscope. The 60 mm dishes were examined at 6–7 and 14–16 days; 90 mm dishes at 8–12 and 20–21 days. Several dishes from each set were chosen to monitor the development of cultures and were examined daily. Negative controls (non-inoculated dishes filled with the same media) were also examined at the same times.

After this initial sampling, the saline water in the aquarium was replaced with PJ medium. The aquarium was aerated and left to incubate for 1 month. At the end of the month, the salinity in the aquarium was about 6‰. All water from the aquarium was decanted and replaced with fresh PJ medium. After one more month of incubation, the salinity in the aquarium was about 0.5‰. The aquarium was sampled for the second time and samples were inoculated, incubated and examined in the same manner as described above. Then the freshwater in the aquarium was replaced with Millpore-filtered 15‰ seawater from the Øresund. After 2 months the aquarium was again sampled as described above. This time the salinities of 50% and 70‰ were not used because they had shown almost no growth of amoebae in previous experiments, but the number of inoculated dishes in each set was increased to 20 dishes of each size. This final sampling showed considerably fewer species. To avoid omitting species, sampling of the sediment was repeated in a similar manner twice more, at weekly intervals.

Amoebae were identified to genera and (when possible) to species using an appropriate set of methods (including cloning, culturing, and TEM studies when required). Many amoeba species demonstrated considerable polymorphism under different levels of salinity, especially at the limits of tolerance and some of them (especially Vannellidae) could not be reliably recognized with certainty at abnormal salinity. To make the results robust, only clearly recognizable species, identified at any salinity without doubt, were taken into account in the analysis.

Results

In total, 28 confidently-distinguishable amoeba species were recorded (Table 1, Figs 4–13), which is close to the usual number of species recorded from a single sample in this habitat (Smirnov and Thar 2003, 2004).