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Observations on Fungal Infection of the Ovary of Laboratory-Cultured *Daphnia magna*

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Daphnia magna is an important test species for aquatic ecotoxicological studies. This is due both to its role in freshwater trophic chains and to its small size, easy reproduction and low cost maintenance in the laboratory. Rearing of D. magna must protect the animals from any external contaminant (i.e., pathogenic organisms or environmental pollutants) which might adversely affect the experimental results. Among the pathogenic microorganisms, the fungi are common infectious agents in fishes (Richards 1978), and they should also be controlled carefully in laboratory-reared aquatic arthropods (Codreanu and Codreanu-Balcescu 1981).

During an in vivo acute toxicity study on marine and freshwater crustaceans (Macrì et al. 1988) a low reproductive index in a strain of D. magna was observed due to infection by a pathogenic fungus. The following note reports our observations on such an outbreak and suggests causal factors thought to contribute to fungal infections.

MATERIALS AND METHODS

The D. magna strain was obtained from the Laboratory of Parasitology collection (Istituto Superiore di Sanità, Rome), was kept in tap water and fed with commercial brewer's yeast and unicellular algae (Chlorella sp).

The test procedure followed the European Economic Community experimental protocol (1979). The maintenance conditions were as follows: temperature = 21°C; photoperiod of L/D = 14/10 hr; light = 1,000 lux; culture water = 70% tap water and 30% distilled water; O_2 (aeration by Rena 101, Euroaquarium, Bologna, Italy)= 5.8-6.0 mg/L (oxygen probe, Hanna Instruments 8543, Padova, Italy); pH = 8.5 (Hanna Instruments 8424, Padova, Italy); hardness = 250 mg/L $CaCO_3$ (Hanna Instruments 8033, Padova, Italy).

The test procedure was as follows: a sufficient (n = 50) number of

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daphniae showing eggs in the dorsal brood chamber were removed from the stock culture and placed in a glass container and maintained under the environmental conditions described above. Neonates 24 hr old were isolated and allowed to grow together until the laying of eggs. The following generation of daphnids was then collected and employed for the test when about 18 hr old.

The daphniae were observed under a dissection microscope (Zeiss, Stemi-SV8). Infected daphniae were isolated in another glass container and their eggs were removed aseptically and seeded on Dextrose Sabouraud Agar (DSA) plates (Oxoid, Basingstoke, UK). The plates were incubated at 26°C for 4-5 d and the growth of characteristic colonies on DSA was observed. Fresh and methylene blue-stained preparations of both eggs and daphnids were examined under optical microscope (Nikon, Microphot-FX).

RESULTS AND DISCUSSION

In the culture containers 11/50 daphniae (about 20%) were infected. Microscopic examination of the dorsal brood chamber revealed orange-colored and moderately swollen eggs on whose surface opaquely white filaments were detected ; in our strain the usual size of eggs laid within 24 hr is 0.25-0.30 mm, while infected eggs ranged 0.30-0.35 mm. (Figure 1). It was noted that daphnids were born from only about 10% of infected eggs. Such daphnids were completely invaded by the fungus and subsequently died (Figure 2).

To avoid a further spread of the infection, the culture was completely eliminated, as it was impossible to perform the ecotoxicity test. The study was postponed after disinfection of the glass culture tanks and of their surroundings with UV rays and alkyl-chlorhydrate ammino-ethyl-glycine (Tego 103 G, TH.Goldschmidt AG, Essen, Germany).

Under the optical microscope a characteristic type of mould with unseptated filamentous thallus was observed. This is an important feature to identify Saprolegniaceae, a family of animal and plant parasites. The pathogenic fungus was tentatively identified as belonging to Saprolegnia sp. which are typical inhabitants of freshwater fish ponds (Kabata 1985), often parasitizing fish (Sieburth 1979). It was not possible to identify precisely the fungal strain or to perform an experimental infection, due to the low amount of infectious material available.

In D. magna cultures infected by Aphanomyces daphniae (Saprolegniaceae), the entire body of the crustacean was invaded (Seymour et al. 1984). Other fungi may also infect the whole body of D. magna. For example, Metschnikowia sp., a yeast genus, can invade, through active multiplication, all the haemocoelic spaces of the host (Codreanu and Codreanu-Balcescu 1981).

An interesting feature of this described outbreak was its apparent selectivity. Based on apparent infections in D. magna brood cham-

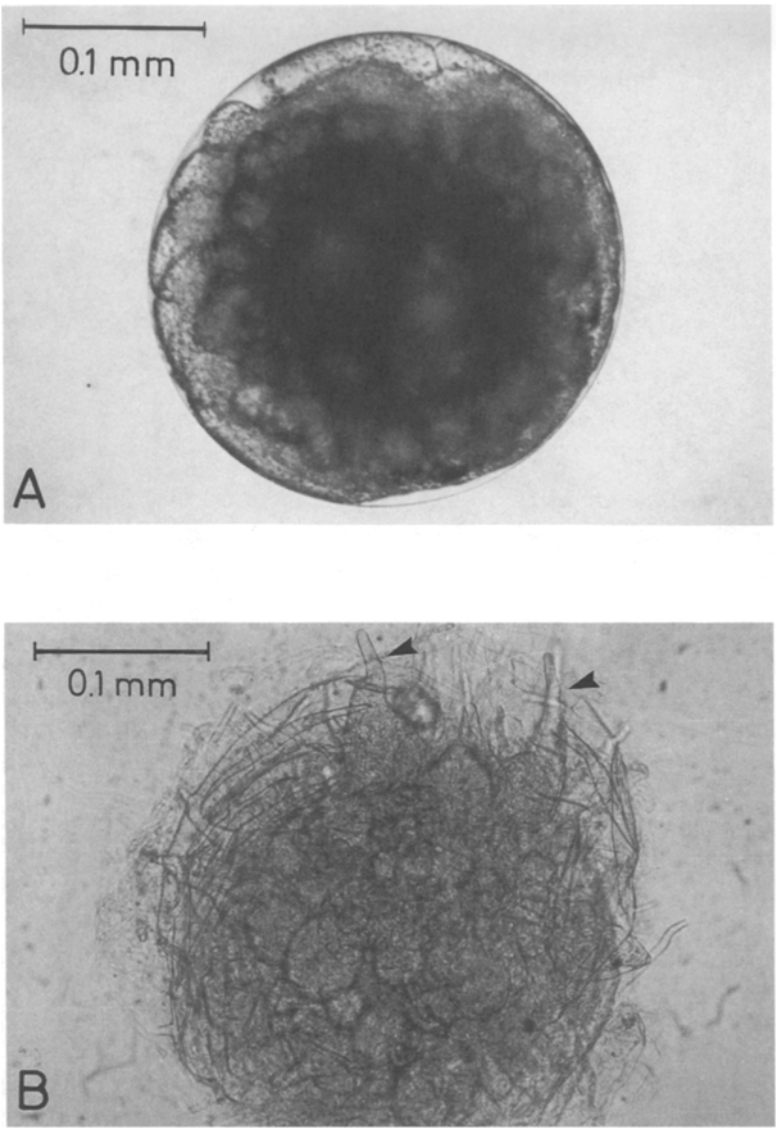

Figure 1. Eggs of Daphnia magna laid in the dorsal brood chamber within 24 hr and isolated for observation under dissecting microscope.
a) Normal egg
b) Egg filled by fungal hyphae (arrows).

bers, the infectious fungi may have been taken up by the female from the culture environment, and transferred internally resulting in infections of the egg. In fact the adult daphniae appeared healthy and were able to survive but unable to reproduce. While some Saprolegniaceae are saprobes, others infect the ova and embryos of molluscs and crustaceans (Sieburth 1979). Goettel (1987)

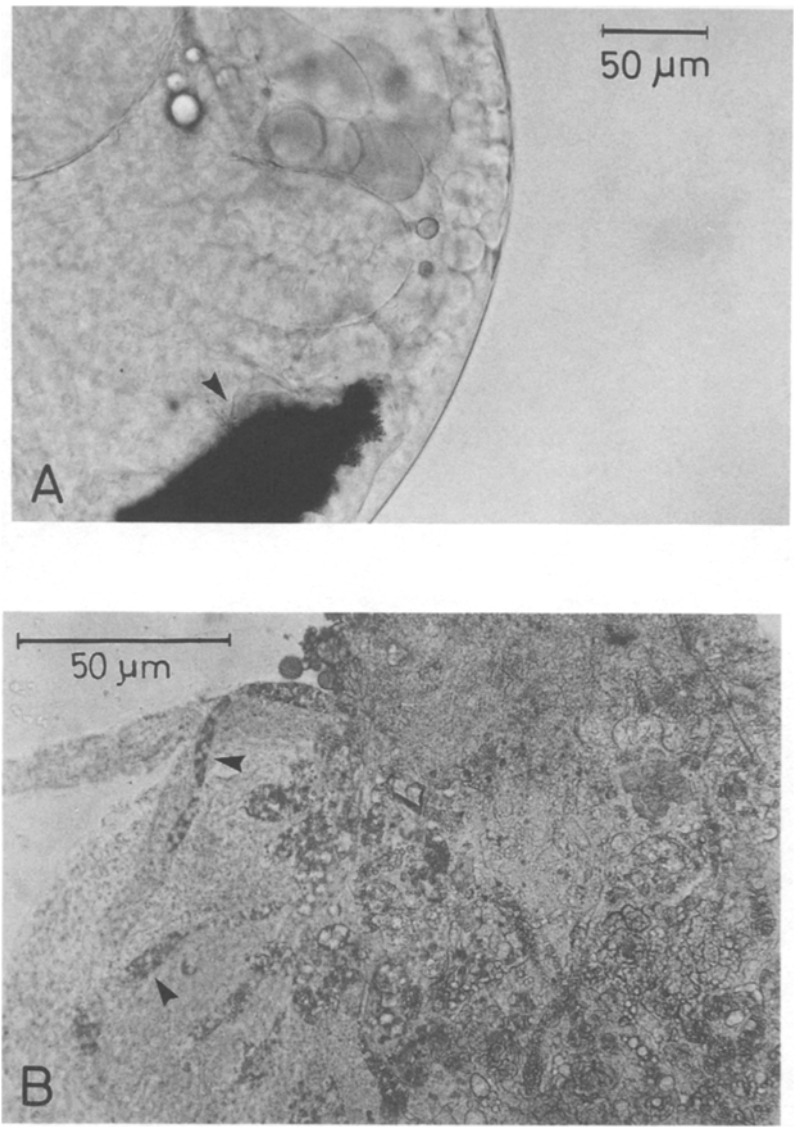

Figure 2. Cephalic portions of neonate daphnids.
a) Normal; eye is visible (arrow).
b) Showing invasion by fungal hyphae (arrows).

observed a high incidence of Saprolegniales infection in mosquitoes which could be related to stressful laboratory conditions. In agreement with Seymour et al. (1984) we observed that the infection could be favored by the presence of dead algae on bottoms of containers, probably due to suboptimal lighting and insufficient oxygen in the original breeding culture. It can be supposed that in such an environment the daphniae were already infected by the fun-

gus, although not visibly so. The change of the environmental conditions, from the original collection to the experimental culture, may have induced a stress in the animal population stimulating the infection to develop.

Careful environmental control of D. magna cultures should include good aeration and the frequent removal of detritus from the tanks or, preferably, the daily transfer of individuals to clean water and containers. Such procedures required for protection from fungal infection as these agents can both destroy the cultures and lead to incorrect experimental results, expecially with regard to reproductive toxicity tests (Cabridenc 1987). It might be possible that insufficient oxygen and high presence of detritus would occur also in field studies. Such situations would be more difficult to control than the laboratory studies, and fungal infection could easily spread among the experimental animals.

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