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Short communication

Culture of the cladoceran *Moina macrocopa*: Mortality associated with flagellate infection

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ABSTRACT

Cladocerans are important food animals in aquaculture, key grazers in freshwater ecosystems, and model animals for ecotoxicological investigations. Their epibiont community, extensively studied in *Daphnia*, includes filamentous bacteria, fungi, algae, peritrich ciliates, and rotifers; although epibionts are usually benign, heavy infections can be detrimental. During our laboratory culture of female *Moina macrocopa* Straus, we observed a novel flagellate infection associated with mortality. At day 10, all *M. macrocopa* were alive in uninfected cultures, whereas in untreated infected cultures, the survival was significantly lower: only 26% of cladocerans were alive. In infected cultures treated with humic substances (as 25 mg L^{-1} dissolved organic carbon), mortalities were comparable to those in the untreated infected cultures; in contrast, in the infected cultures treated with 4 g L^{-1} sea salt, mortalities were arrested, and 76% of the *M. macrocopa* were alive at day 10. Moribund cladocerans were transparent, had empty digestive tracts, and greatly reduced motor activity. Free-swimming flagellates moved forward with a wobbling motion, rotating around their long axis; they also attached to cladoceran tissue, the Petri dish, and the glass slide, by the tip of their posterior flagellum. Flagellates preserved for scanning electron microscopy were 6.9 ± 0.7 μm long and 2.1 ± 0.3 μm wide, with a short anterior flagellum (6.8 ± 1.1 μm) and long posterior flagellum (14.1 ± 1.5 μm). Multi-functionality of a flagellum, for locomotion and adhesion, is relatively rare, and previously reported from genera within the Kinetoplastea, suggesting that the flagellate on *M. macrocopa* may belong to this group. To combat flagellate mass occurrence in *Moina* cultures, we recommend a treatment with 4 g L^{-1} sea salt.

1. Introduction

Cladocerans of the genus *Moina*, and *Moina macrocopa* Straus in particular, are progressively important in aquaculture and ecotoxicology. *Moina* spp. are increasingly used as food for larval and post-larval rearing of crustaceans (Alam et al., 1993) and teleost fish in culture (He et al., 2001; Ingram, 2009; Peña-Aguado et al., 2009). Due to a relatively high protein and nutrient content, *Moina* spp. is a superior live food compared to *Artemia* (Alam et al., 1993; Loh et al., 2012). Furthermore, the use of freshwater zooplankton, such as *M. macrocopa*, may be more convenient for feeding freshwater species than is use of saltwater *Artemia* (Alam et al., 1993; Loh et al., 2012).

Although *Moina* is widely distributed, from temperate to tropical regions, commercial scale quantities of this cladoceran are not easily obtained from natural habitats (Loh et al., 2013). Mass cultivation for live feed has been successful, and *Moina* tolerates low oxygen and high ammonia, reproduces rapidly, and grows rapidly on a range of food sources (Loh et al., 2013). There continues to be considerable focus on investigating different foods for mass culture of *M. macrocopa* (Kang et al., 2006; Loh et al., 2009, 2013).

In the laboratory, *Moina* spp., and *Daphnia magna* Straus are widely used model animals in ecotoxicity testing of synthetic and natural xenobiotics. Of particular note is that *Moina* sp. may be used as the replacement for *Daphnia* in regions where the latter does not occur naturally (Ferrão-Filho et al., 2010; Mano et al., 2010; Sarma and Nandini, 2006).

The successful and reliable culture of cladocerans as food for aquaculture species is dependent on many factors, including maintenance of healthy stocks, and effective diagnosis of disease-causing organisms such as parasites. Cladocerans are hosts to a diversity of epibiont taxa, including filamentous bacteria, fungi, algae, peritrich ciliates, and rotifers (Ebert, 2005; Green, 1974). Heavy coatings of epibionts can be a weight burden, increase drag (Gilbert and Schröder, 2003), reduce population growth (Green, 1974; Stirnadel and Ebert, 1997), and those on the thoracic limbs can lower the resistance of their host to oxygen deficiency (Pacuad, 1939). Among the parasitic taxa infecting cladocerans are bacteria, fungi, microsporidia, cestodes, and nematodes, which may cause

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behavioral changes (Decaestecker et al., 2005; Makrushin, 2010) and reduced egg production (Green, 1974; Stirnadel and Ebert, 1997).

Although flagellates have not been reported from cladocerans, they do infect copepods, another group of small freshwater crustaceans (Hitchen, 1974), and thus they might be found on cladocerans. *Cephalothamnium cyclopum* Stein (incertae sedis Kinetoplastea) forms stalked colonies on the copepod *Cyclops* sp.; one flagellum attached to a communally-secreted stalk, the other is used in food gathering (Hitchen, 1974).

While the epibiont and parasite fauna of cladocerans is well known for *Daphnia* (Ebert, 2005), the fauna of the increasingly important genus *Moina* is little known. Since the classical study by Green (1974), identifying a variety of epibionts and parasites on *M. macrocopa*, such as *Megachytrium* sp., *Chloranigiella epizooticum* Korschikoff, *Pansporella perplexa* Chatton, *Epistylis helenae* Green, and *Brachionus rubens* Ehrenberg, there appears to have been only one report of a parasite in *Moina*, namely the microsporidia *Gurleya* sp. in *M. macrocopa* (Makrushin, 2010). We now extend knowledge of pathogenic infections in *Moina* spp. by reporting our light microscopy and scanning electron microscopy observations on the dense infections of flagellates associated with mortality of cultured *M. macrocopa* used in xenobiotic exposure experiments.

2. Materials and methods

2.1. Stress ecology studies and source of Moina

The background for the present investigation was our maintenance of cultures of *M. macrocopa* for stress ecology studies, in which we aimed to determine whether the heritage of cross tolerance was epigenetically controlled and based on DNA methylation in the presence of humic substances. To pursue this, in xenobiotic experiments, we pre-exposed *Moina* to humic substances, and then tested their cross tolerance against sea salt (following Suhett et al. (2011)). During these xenobiotic experiments, some cultures became infected with flagellates, allowing us the opportunity to study them, and their response to DOC and salt.

M. macrocopa (Fig. 1a) is a characteristic inhabitant of small, usually ephemeral, water bodies from temperate to tropical regions, which are often rich in dissolved organic carbon (Petrusek, 2002). Our clone was originally isolated from a puddle in Rio de Janeiro, Brazil (Elmoor-Loureiro et al., 2010), and has been successfully used since then in life table and cross tolerance studies in stress ecology (Hofmann et al., 2012; Suhett et al., 2011), and in a recent DNA methylation study (Menzel et al., 2011).

2.2. Maintenance of cladoceran cultures and cross tolerance experiments

The stock culture was maintained in artificial *Daphnia* medium (Klüttgen et al., 1994). Only neonates of the 3rd generation under identical laboratory conditions were used for the experiments. *M. macrocopa* reproduces partheogenetically under stable laboratory conditions (contrasting with sexual reproduction in times of stress), and all offspring from this asexual reproduction were female. *M. macrocopa* was fed daily, ad libitum, with the coccal green algae *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J. Kristiansen & O.M. Skulberg.

Each xenobiotic experiment was initiated with 10 replicates, in each of which were 10 *M. macrocopa* in a 200 ml Erlenmeyer flask. During the experiments, although some replicates were lost, there was always a minimum of 8 replicates for each experiment; (thus the number of live cladocerans expected at each time point per experiment was 80, 90 or 100, assuming no mortalities). The flasks were kept in a temperature-controlled room at 20 ± 1 °C, and illuminated by cool white light in a 14:10 h light:dark rhythm. Every second day, the number of live and

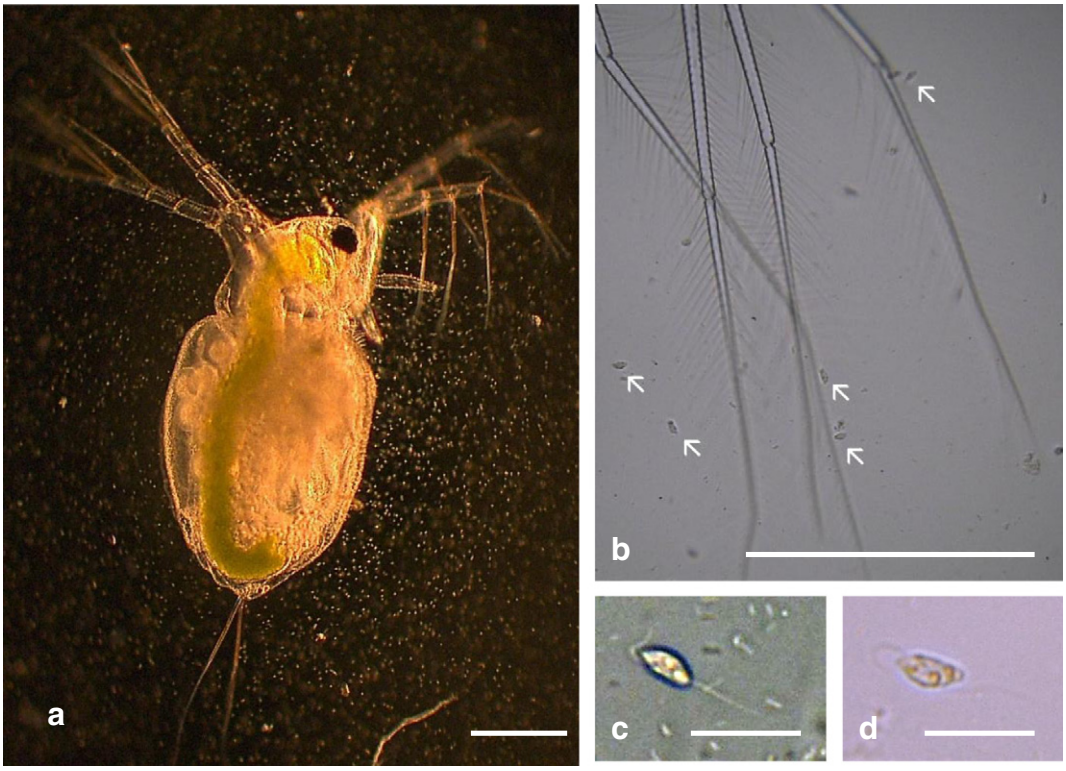

Fig. 1. Light micrographs of *Moina macrocopa* and live bodonid flagellates from the *M. macrocopa* cultures. (a) Healthy parthenogenetic *Moina macrocopa* female, (b) posterior part of the second antenna of the cladoceran, arrows indicate the flagellates, (c, d) two flagellates showing the short whiplash anterior flagellum and the long posterior/recurrent flagellum, note also the yellow-brown refractile inclusions. Scale bars = 100 μm (a, b) and 10 μm (c, d).