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Research Brief

New technique for collecting eggs from monogenean parasites

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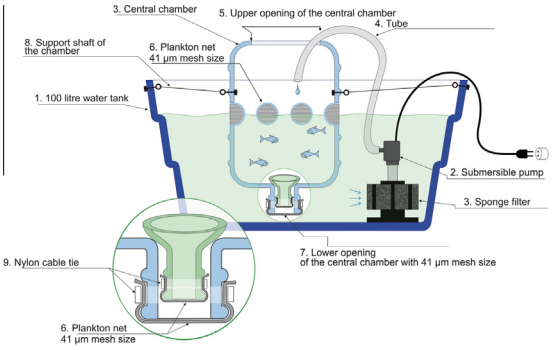
HIGHLIGHTS

- A new method for collecting monogenean eggs is proposed.
- Use of this technique may lead to a better understanding of parasite's biology.
- It can be used in a wide range of biological studies.
- It offers controlled environmental conditions to fish under long-term studies.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel and easily constructed apparatus for collecting monogenean eggs released by the parasites into the water is described and illustrated. Use of this technique may lead to a better understanding of the parasite's biology, which, in turn, may lead to the improvement of parasite management strategies in fish farms where monogeneans are potentially harmful to their hosts. The technique is also useful for studies of eggs or free-living stages of other fish parasites.

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1. Introduction

With direct, water-based life cycles, monogenean parasites are able to release a variable amount of eggs into the water probably mediated by parasite, host and environmental pressures regulating the reproductive processes. Under culture conditions, these parasites may proliferate easily and therefore can be considered as one of the most important parasite group with potential to generate significant economical impact in Brazilian fish farms (Moraes and Martins, 2004).

Parasite-associated costs can be reduced by implementation of parasite management strategies, which rely on a good understanding of parasite and host (Mooney et al., 2008). With this in mind, the present authors aimed to characterize a new method for collecting monogenean eggs. This technique can be easily used in a wide range of biological studies, besides offering controlled environmental conditions to fish under long-term studies.

2. Description of the apparatus

The experimental unit (Fig. 1) was composed of a 100 l water tank (1) provided with a submersed pump (2) linked to a sponge filter (3) so the water was constantly being filtered and, with the

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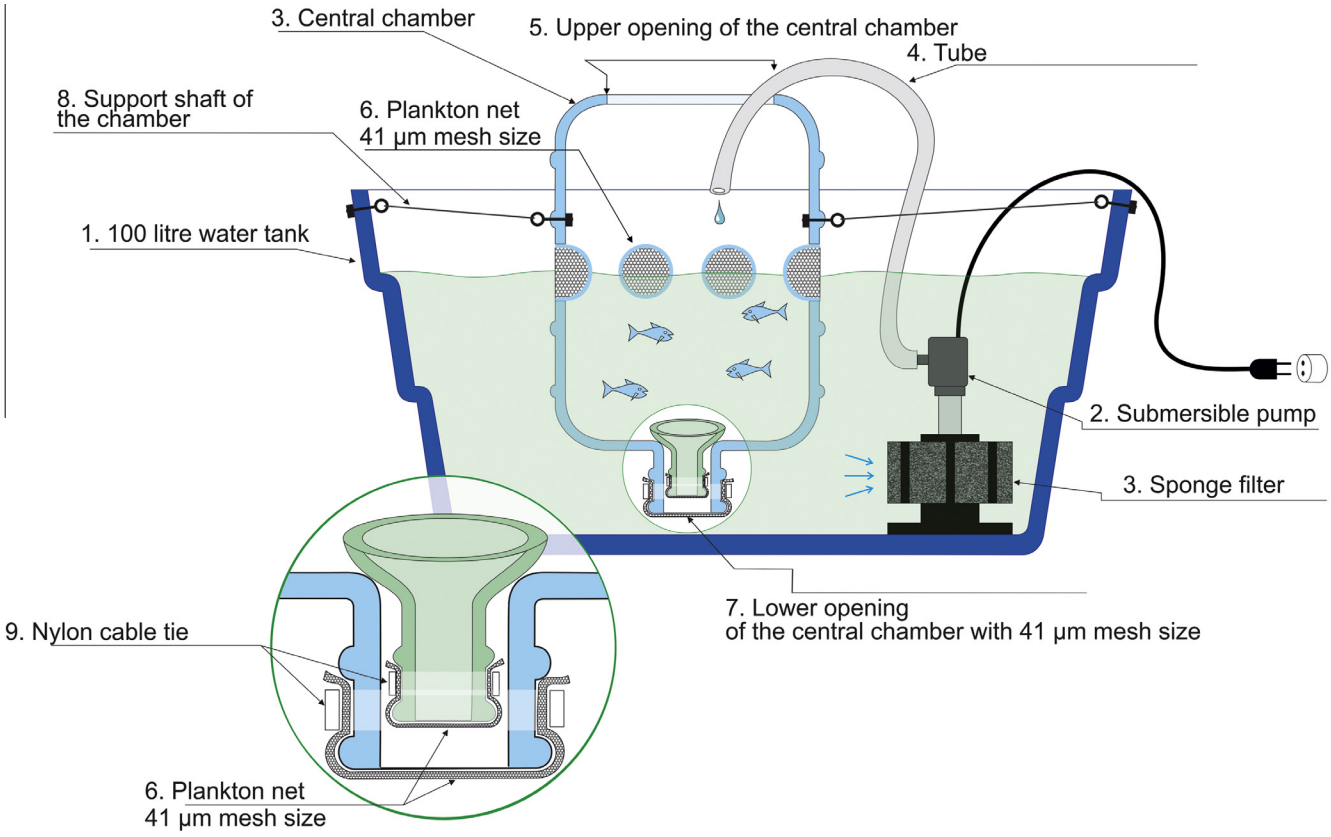

Fig. 1. Schematic drawing of the technique.

aid of a tube connected to the filter (4), sent to the interior of the central chamber (5), a 20 l plastic bottle with eight circular openings arranged horizontally in the central part of the chamber and covered with a plankton net 41 µm mesh size (6). The chamber was kept in place inside the 100 l water tank with the aid of a support shaft (8) that can be made of polyvinyl chloride (pvc) electric wire. The lower opening of the chamber (7) was fitted with a plastic funnel, here called inspection apparatus, whose neck was covered by plankton net (41 µm mesh size) (6). The plastic funnel may be made with the top of a liter plastic bottle. To make sure the plastic funnel was well adjusted and no eggs were lost to the tank, another plankton mesh was added at the bottom of the chamber (main filter mesh) (7). Nylon cable ties (9) were used to attach both the plankton meshes (plastic funnel and lower opening of the chamber) in their respective, while the meshes of the eight central circular openings were securely glued.

3. Test of the apparatus

Parasitized freshwater fish *Rhamdia quelen* (Quoy and Gaimard, 1824) (Heptapteridae) were collected from a local fish farm in February 2012. All procedures were carried out according to the international practices for animal use and care under the control of an internal committee (UFSC/23080.029980/2009-21).

Thirty-six fish with 9.74 ± 0.76 cm and 6.85 ± 1.84 g were set into nine experimental units. Four fish were placed in each central chamber for ten days. No deaths were recorded during the time and water quality parameters were as follows: ammonia 0.31 ± 0.11 mg/l, water temperature 23.67 ± 0.96 °C, pH 8.33 ± 0.59, alkalinity 40.0 mg/l and dissolved oxygen 9.22 ± 0.58 mg/l. Water quality parameters were within the accepted welfare range for the fish species herein studied, according to Gomes et al. (2000); Piedras et al. (2004).

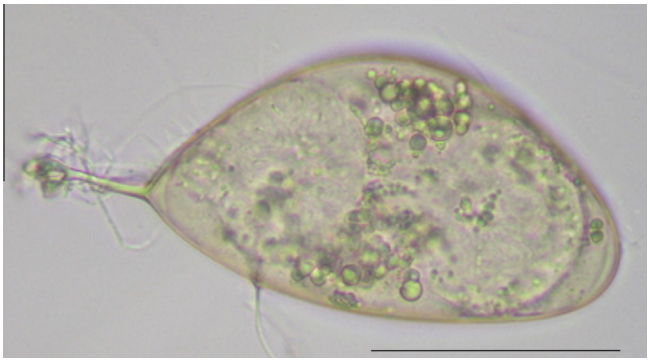

Fig. 2. Egg of *Aphanoblastella mastigatus* (Suriano, 1986). Bar: 50 µm.

After 12 h the inspection apparatus was removed and its content was thoroughly washed into a Petri dish in order to verify the presence of monogenean eggs. By the end of the first 24 h, fish were momentarily transferred to the main tank and the central chamber was suspended and thoroughly washed (this includes netting covering the circular openings) to concentrate all eggs in the main filter, which was also washed into a Petri dish for analysis.

The meshes from the lower opening of the central chamber were then replaced and fish were relocated to the central chamber where they kept for ten more days in order to evaluate the filter efficacy of the apparatus in prolonged use.

An analysis of the filtered content revealed the presence of eggs of the monogenean *Aphanoblastella mastigatus* (Suriano, 1986) (Dactylogyridae). The eggs of this parasite were identified by their size 96.8 ± 8.9 (84–116; *n*: 40) length by 46.6 ± 4.2 (44–63; *n*: 40) µm width, shape and by the presence of a long appendage at one

end of the egg (Fig. 2). These were incubated in Petri dishes and after 72 h oncomiracidia began to emerge from the eggs.

The use of the inspection apparatus (the removable plastic funnel) brings an interesting advantage which is the possibility to remove it from the system (without causing any damage to it) to make a detailed analysis of its content (as for example feces, eggs, free-living infective stages) and later put it back into the chamber. This provides the opportunity to determine when parasite egg laying begins and to determine whether or not fishes are infected.

The eight circular openings arranged horizontally in the central part of the chamber serve two main purposes, first to ensure adequate water exchange with the outer filtration system, thus maintaining adequate water quality in the chamber, especially adequate oxygen levels. Second, they extend above water level to prevent water overflow in case of inadequate water flow in the bottom net, event in which most of the eggs would be lost. Nevertheless, the netting was carefully washed during every inspection of the filtered material.

4. Discussion

The following methods for collecting monogenean eggs were used by previous authors: keeping the fish in small bags of nylon mesh (Buchmann, 1988); introducing nylon nets into the tank containing infected fishes, so that the eggs become entangled in the nets (Hirazawa et al., 2010); collecting eggs from detached live parasites kept in Petri dishes (Chisholm and Whittington, 2000; Kritsky and Stephens, 2001). All of these techniques may, in fact, succeed in the collection of the eggs. However, they may better expose fish to multiple stresses such as handling and/or poor water quality and may be, probably, not so efficient when long-term studies are proposed.

The new technique herein described can be therefore considered a useful tool for several parasitological studies concerning the biology, life cycle, experimental infection as well as treatment of fish parasites. It is a practical technique, which is able to provide controlled environmental condition for long-term parasitological studies.

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