



First record of an aquatic oligochaete infesting fish

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ABSTRACT

This case represents the first documented report of aquatic oligochaetes (*Pristina aequisetata*) infesting fish. Oligochaetes are common and ubiquitous in aquatic environments, but parasitic (and symbiotic) species are extremely rare with a few species occurring in frogs. During a disease surveillance project, live oligochaetes were observed in fresh preparations of gills of the Australian freshwater Murray cod (*Maccullochella peelii*) that had been reared in a recirculating aquaculture system. Large numbers of oligochaetes were also found in detritus from the biofiltration system of the tanks. In autopsied fish, patches of filaments showed marked and diffuse hyperplasia, goblet cell metaplasia and mainly mononuclear infiltrate. This infestation may have caused sufficient damage to compromise the health of the fish and even death, considering that heightened mortality had occurred prior to their discovery. This infestation was considered not a case of parasitism but rather an opportunistic colonisation event triggered by a number of factors including the presence of a large population of *P. aequisetata* within the recirculating aquaculture system and unhealthy (or stressed) fish that could not ward off infestation.

1. Introduction

Oligochaetes (Annelida: Clitellata) are common and ubiquitous in aquatic environments with nearly 200 limnic oligochaete species known from Australia (Pinder 2010). Despite this diversity, parasitic (and symbiotic) oligochaetes are extremely rare; a few species occurring in the ureters of frogs (Harman 1971).

Recirculating aquaculture systems (RAS) have long been used to rear fish in a controlled environment. In these systems fish are typically held at moderate to high densities in tanks. The wastewater discharged from the culture tanks is partially re-used after being mechanically filtered to remove solid wastes and biologically filtered to manage metabolic wastes (nitrogen and phosphorous) (Timmons and Losordo 1994; Ebeling and Timmons 2012). The surfaces of the media within biological filters and the sludge/detritus/sediment therein contain complex communities of organisms, which includes microbes, especially those associated with utilisation of nitrogenous wastes, protozoans, rotifers, gastrotriches, oligotriches, nematodes and oligochaetes (Curds and Hawkes 1975; Learner and Chawner 1998; Fried et al., 2000; Sugita et al., 2005).

Murray cod (*Maccullochella peelii*) (Percichthyidae) is a freshwater

cod-like species from south-eastern Australia that is highly valued by consumers. Murray cod is commercially farmed in recirculating aquaculture systems, cages and dams for domestic and export markets (Ingram et al., 2005a; Gooley and Bailey 2006).

We describe the first record of an infestation of aquatic oligochaetes, *Pristina aequisetata* Bourne 1891 (Naididae), amongst gill filaments of a fish, Murray cod.

2. Materials and methods

Fish infested with oligochaetes were collected from a commercial farm in southern New South Wales, Australia, that was growing Murray cod in an intensive recirculating aquaculture system. Samples of Murray cod were collected on August 20, 2013 during a disease surveillance project (Bradley et al., 2014). Farms participating in the project were visited on a regular schedule and a selection of fish taken for gross and histological examination. Fresh samples of gill tissue and skin mucus were examined at the time of collection under magnifications of 40–400× to check for presence of parasites. At this stage, micrographs of live specimens were taken with a Marlin F-046C camera (Allied Vision Technologies, Germany). Sampled fish were anaesthetised in AQUI-S®

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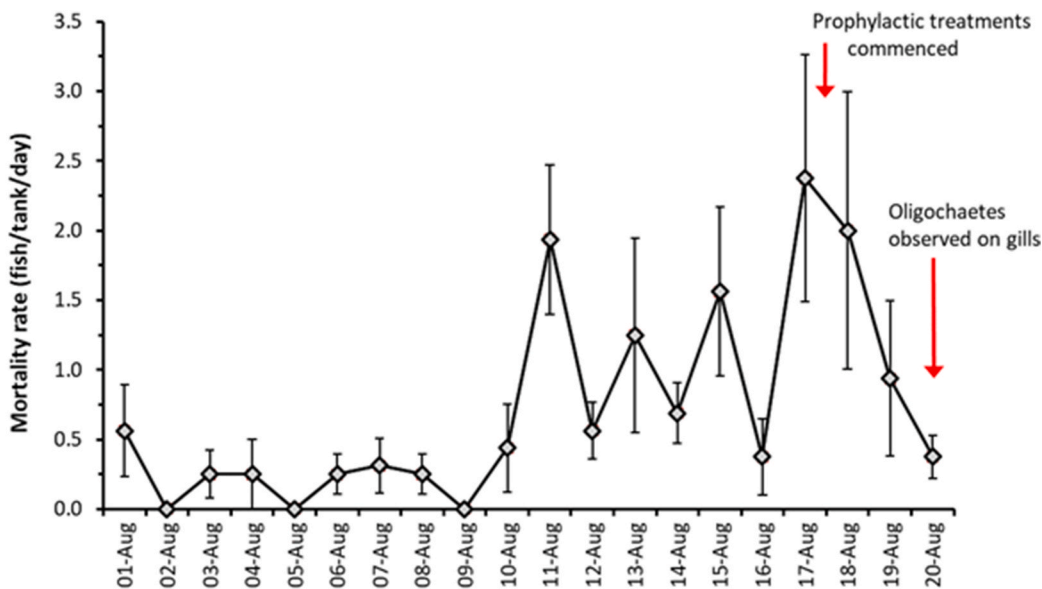


Fig. 1. Fish mortality rate (fish/tank/day) for 16 tanks in a Murray cod recirculating aquaculture system infested with *Pristina aequisetata* (values represent mean and standard error).

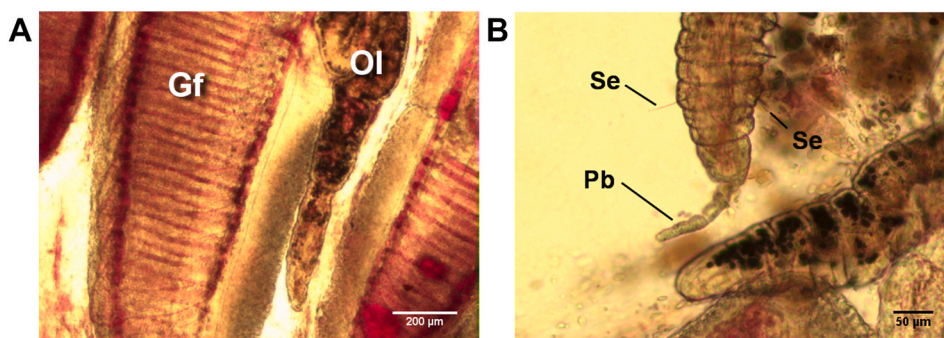


Fig. 2. *Pristina aequisetata* between gill filaments of Murray cod. (a) Low magnification showing gill filaments (Gf) and oligochaete (Ol). (b) Anterior end showing distinctive proboscis (Pb) and segments with setae (Se).

(Aqui-S, New Zealand) (active ingredient 10% eugenol) then euthanised. Fish that were euthanised were autopsied and samples of gill, skin, eye, brain, kidney, pancreas, heart, spleen, stomach and intestine were fixed in 10% buffered formalin for histology. Fixed tissues were sectioned and stained with haematoxylin and eosin (H&E) in routine fashion.

3. Results and discussion

Sampled fish were 195–230 mm in length and 92–144 g in weight. Fish mortality in the tanks was generally <0.5 fish/tank/day (mean value for 16 tanks), but since 9 August, mortality had increased dramatically, and 15 deaths were recorded in one tank on one day (Fig. 1). In the days following the peak in mortality on the 17 August, the tanks were treated with formalin and salt to control parasites and Chloramine T (a sulphonamide antibacterial agent and disinfectant).

Water temperature, dissolved oxygen and pH in the tanks in the three weeks prior was 21.9–24 °C (mean 23.0 °C), 4.0–7.5 ppm (5.7 ppm) and 5–7.3 (mean 6.5), respectively.

Live organisms, which were identified as oligochaetes due to the presence of characteristic body segments bearing setae, were observed in fresh preparations of gill biopsies from two (Fish 1 and 2) of the three fish sampled, one with six specimens present and the other with two specimens. The oligochaetes could be seen actively moving in the mucus

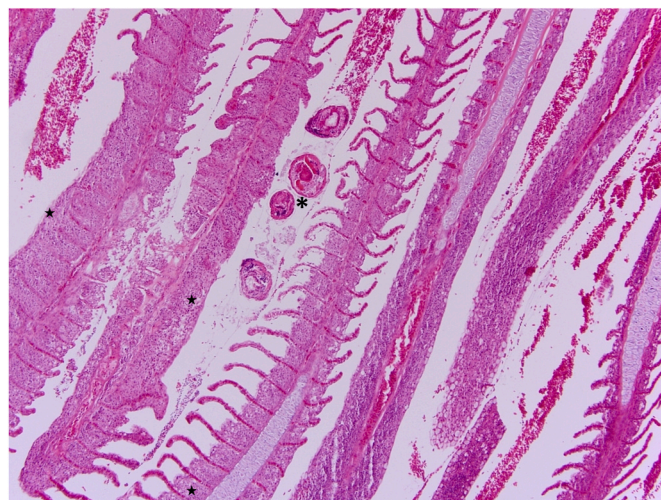


Fig. 3. Stained section of gill tissue showing oligochaete profiles with cuticle, cuticular muscle and coelomic organs (asterix) within mucus and debris, between gill lamellae showing epithelial hyperplasia, goblet cell metaplasia and inflammatory infiltration of the lamina propria (stars) (400× magnification).

and debris between gill filaments. No other organisms were observed in fresh wet preparations of gills and skin scrapes. The oligochaetes were identified as a species of *Pristina* (Naididae) based on size, the presence of a proboscis and distribution and form of the setae (Fig. 2).

Large numbers of oligochaetes were subsequently found in detritus collected from the biofiltration system of the tanks. These were identified as *Pristina aequisetata*, presumably the same species as observed in gill samples, and *Dero furcata* (Müller) (Pinder, 2010). *Pristina aequisetata* is a widespread cosmopolitan epibenthic species found throughout Australia (Pinder 2001) and globally (Brinkhurst and Jamieson 1971).

Tissues of five fish were examined histologically. Oligochaetes were observed between filaments in gill sections from three fish. Multifocally there was marked epithelial hyperplasia and goblet cell metaplasia with mainly mononuclear cell inflammation in gill lamellae of affected fish (Fig. 3). These were mostly associated with oligochaete profiles present within mucus and debris between the lamellae. Other, smaller foci of mild epithelial hyperplasia were present and there were scattered large epithelial cells with granular cytoplasm (epitheliocystis).

It is not clear that the response observed in the gills was due simply to the presence of *P. aequisetata* amongst the gills, or *P. aequisetata* feeding on gill tissue. The response by the fish to the infestation in the gills is similar that elicited by other gill parasites. However, it is noted that no other gill parasites were observed in either fresh preparations or sectioned material that could have elicited these responses.

The presence of *P. aequisetata* on the gills of Murray cod may have caused sufficient damage to compromise the health of the fish and even death. Indeed, fish mortality in the tanks had increased dramatically prior to the discovery of the oligochaetes (Fig. 1). However, we consider that this infestation was not a case of parasitism but rather a rare opportunistic colonisation event triggered by a numbers of factors including the presence of a large population of *P. aequisetata* within the recirculating aquaculture system and unhealthy (or stressed) fish that could not ward off invasion. This and other oligochaete species regularly occur in the filter beds of sewage works (Learner 1979; Learner and Chawner 1998) so conditions in the recirculating aquaculture system at the time of the infestation may have been conducive to their proliferation. Since Naidids consume organisms associated with detritus (bacteria and algae) (Harper 1981), a build-up of uneaten food and faecal matter in the recirculating aquaculture system may have suited *P. aequisetata*. Poor water quality, which occurs under these conditions, has been associated with prevalence of other parasites in recirculating aquaculture systems, such as the ciliated protozoan *Trichodina* (Madsen et al., 2000).

This case is the first report of an aquatic oligochaete infesting fish. While this record may not be a case of parasitism, parasitic oligochaetes are known but extremely rare. A number of species of endoparasitic (symbiotic) naidids have been reported from frogs (Goodchild 1951; Harman 1971). Some enchytraeid and phreodrilid species are known to inhabit (as a commensal) the gill chamber of higher crustaceans (Brinkhurst 1991). Parasitism is more widespread in other clitellate annelids. Leeches (Hirudinea), such as *Piscicola* spp., are regularly reported from both freshwater and marine fishes (Hoffman and Meyer 1974; Rohde 1993) and branchiobdellids can be ectoparasites of crayfish (Skelton et al., 2013). Over 80 polychaete species are associated with 253 different hosts, but only one species, *Ichthyotomus sanguinarius*, is a parasite of fish (Ansell et al., 1998).

Opportunistic colonisation of fish by apparently non-parasitic (or species capable of facultative parasitism) organisms are reported throughout the literature, and often involves species present in wastewater treatment systems, such as recirculating aquaculture systems. Aquatic mites have been associated with pathological effects on fish (Fain and Lambrechts 1987), and one species, *Histiostoma papillata*, found on the fins and gills of juvenile Murray cod, was suspected of causing injuries leading to mortality (Halliday and Collins 2002). The sessile peritrich *Epistylis* has been observed on the body surface of Murray cod reared in a recirculating aquaculture systems (Halliday and

Collins 2002; Ingram et al., 2005b). Most sessile peritrichs are filter-feeding bacterivores and their occurrence is usually associated with poor water quality and high organic loading (Curds 1982; Foissner and Berger 1996).

3.1. Options for management

Fish infested with *P. aequisetata* may be treated with approved chemicals used for other external metazoan parasites, such as formaldehyde and salt. Long-term management should focus on managing water quality and reducing nutrient loading in culture systems to inhibit the build-up of *P. aequisetata* populations. Reducing habitats preferred by *P. aequisetata* by regular removal of sediments and detritus is also recommended.

Declaration of competing interest

None.

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