

Observations on the health of *Tandanus tropicanus* (Teleostei: Plotosidae) from an Australian river system

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ABSTRACT

Wild Wet Tropics tandan *Tandanus tropicanus* were collected from the Bloomfield River, Queensland, for examination by histopathology and bacteriology. This provided an opportunity to establish baseline information on the general health and parasite fauna of native freshwater catfish in a pristine river. Histology of gill tissue revealed epitheliocystis in one fish. This is the first report of epitheliocystis in *T. tropicanus*. Bacterial culture showed light growth of bacteria from the kidney of only two fish, and these were identified as *Aeromonas veronii*, *A. jandaei* and *Bacillus/Lactobacillus* spp. An unidentified monogenean infection was observed in the gills of four fish, and trematode metacercariae were observed in the extra-ocular tissue of four fish. Nematodes were observed in the tissues of nine fish, and sequence and preliminary phylogenetic analysis of PCR products using an ITS primer suggest that these parasites may be a previously unreported *Contraecum* species.

Key words: wild catfish, histology, fish health, Wet Tropics.

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Introduction

Native freshwater fishes in Australia face many threats from the effects of invasive alien species, exotic disease introductions, climate change and anthropogenic habitat alteration. In particular, the continued importation of large numbers of ornamental fishes into Australia (Stephan and Hobsbawn 2014), in conjunction with Australia's growing aquaculture sector (Buckley and Gilligan 2005; Stephan and Hobsbawn 2014), carries the potential risks of introducing serious disease into wild Australian fish populations. Imported ornamental fishes have previously been linked to the introduction of several fish pathogens into Australia, including *Lernaea cyprinacea* (Hassan *et al.* 2008), gourami iridovirus (Go and Whittington 2006) and atypical *Aeromonas salmonicida* (Humphrey and Ashburner 1993).

Previous attempts to link deteriorations in fish health to anthropogenic environmental changes in Australia have met with little success, due to a lack of baseline reference data. Aquatic organisms, including fish, serve as valuable bio-indicators of ecosystem health (Whitfield and Ellis 2002; Pusey *et al.* 2007; Jia and Chen 2013). Specifically,

fish body condition indices and histological biomarkers (Thilakarathne *et al.* 2007; Liebel *et al.* 2013) have been used to monitor anthropogenic disturbances in ecosystems. However a paucity of knowledge on spatial and temporal variations in fish pathobiology is limiting the extent to which information collected from monitoring programs can be meaningfully interpreted (Cajaraville *et al.* 2000; Whitfield and Elliot 2002). A review by Whitfield and Elliot (2002) states that increased knowledge on health indices of fish communities is needed to assist in successful environmental management.

Benthic species, in particular, are rapid and sensitive indicators of ecosystem stress (Frithsen and Holland 1992). Many catfish species are sensitive to ecosystem changes (de Andrade *et al.* 2004; Azevedo *et al.* 2012), and are used as useful bio-indicators of environmental contamination (Azevedo *et al.* 2012; Pimpão *et al.* 2012; Galeb *et al.* 2013; Harabawy *et al.* 2014). Native Australian catfishes also have the potential to be useful bio-indicators, due to their relative abundance across the Australian continent (Allen *et al.* 2002), and their

benthic habitat. There has been no comprehensive study on the health of any native catfish species in Australia, and therefore there is a lack of baseline reference data on these important freshwater species.

As part of a larger study on the health of native catfish in northern Australia, a number of *T. tropicanus* were collected from the Bloomfield River in northern Queensland. The section of the Bloomfield River in which the catfish were collected is considered to be of high ecological value and effectively unmodified, with no exotic fish species recorded (Burrows 2009; Department of Environment and Resource Management, Queensland Government 2010). This presents a unique opportunity to study the baseline health of a newly described native Australian catfish species (Welsh *et al.* 2014) in a relatively pristine environment.

Materials and Methods

Sampling

Nineteen *T. tropicanus* were collected from one location on the Bloomfield River (15.9868S, 145.2882E, WGS84) in northern Queensland. Juvenile and adult catfish (6.8–207.8 g and 8–30 cm total length) were collected using single-winged fyke nets set overnight on 9th and 10th May, 2014. The catfish were kept in holding nets in the river until they were transported to James Cook University (JCU), Cairns on 11th May, where they were held in well aerated aquaria, until euthanized immediately before examination on 11th and 12th May.

Fish were euthanized by being placed into a prolonged anaesthetic bath of isoeugenol (AquiS™). Sex, weight and total body length measurements were recorded, and fish examined for any external or internal gross abnormalities. For bacterial isolation, pooled kidney and spleen tissues from each individual were homogenised and inoculated onto blood agar (BA) and *Edwardsiella ictaluri* medium (EIM) (Shotts and Waltman 1990). Inoculated plates were couriered in sturdy insulated boxes under ambient temperatures to the Animal Health Laboratories, Department of Agriculture and Food Western Australia, Perth, and incubated at 24°C for 48 to 72 hours. Isolates were identified using matrix-assisted laser desorption ionisation time of flight mass spectrometry (Bruker, Microflex LT MALDI biotyper) and biochemical methods according to Buller (2015).

All major organs were collected from each individual and fixed in 10% neutral buffered formalin for 80 to 100 hours. Following formalin-fixation, tissues containing bone were demineralised in 5% nitric acid for 1 hour before routine histo-processing and embedding in paraffin wax. Five micrometre sections were stained with haematoxylin and eosin (H & E), and selected sections stained with Martius Scarlet Blue (MSB) for fibrin. Where parasites were detected, prevalence of infection was calculated as percentage of hosts examined, with 95% confidence interval estimated assuming a binomial distribution.

DNA isolation

In histological tissue sections where parasites were detected, total DNA was extracted from 4–5 slivers of 5 µm sections

of selected wax block tissues using a Power Soil DNA Kit (MolBio, Carlsbad, California) with some modifications, as described by Yang *et al.* (2012). Briefly, the tissues for DNA extraction were subjected to four cycles of freeze/thaw (liquid nitrogen followed by boiling water) to ensure efficient lysis of tissues before being processed using the Power Soil DNA Kit, according to the manufacturer's protocol. Test reagents with exclusion of tissues served as negative control in each extraction group.

PCR amplification and sequencing

A set of primers, ASITSF 5'-AAC CTG GTT GAT CCT GCC AGT-3' and ASITSR 5'-ATG TGT CTG CAA TTC GC ACT-3', was used to amplify the internal transcribed spacer (ITS) region. The expected PCR product was ~570 bp. The PCR reaction contained 2.5 µl of 10 × Kapa PCR buffer, 3 µl of 25 mM MgCl₂, 1.5 µl of 10nM dNTP's, 10 pM of each primer, 1 unit of KapaTaq (Geneworks, Adelaide, SA), 1 µl of DNA (about 50ng) and 14.9 µl of H₂O. PCR cycling conditions were 1 cycle of 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 sec, 55 °C for 30 sec and 72 °C for 1 min and a final extension of 72 °C for 5 min.

Sequence analysis

The amplicons from the ITS PCR were gel purified using an in-house filter tip method as previously described (Yang *et al.* 2013). Amplicons were sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, California) according to the manufacturer's instructions (with the exception that the annealing temperature was at 58 °C). The results of the sequencing reactions were analysed and edited using Finch TV® v1.4.0. (<http://seq.mc.vanderbilt.edu/dna/html/SoftDetail.html>). Sequences were compared to existing helminth parasite ITS rRNA sequences on GenBank using BLAST searches and aligned with reference sequences using BioEditor (<http://bioeditor.sdsc.edu/download.shtml>).

Phylogenetic analysis

A phylogenetic tree was constructed using the DNA sequences at the ITS region found in this study and sequences of *Contracaecum* spp. and *Hysterothylacium* spp. from GenBank, with *Anisakis simplex* and *Cardicola opisthorchis* as outgroups (Figure 1). Distance estimation was conducted using TREECON (Van de Peer and De Wachter 1994), based on evolutionary distances calculated with the Tamura-Nei model and grouped using Neighbour-Joining. Bootstrap analyses were conducted using 1,000 replicates to assess the reliability of inferred tree topologies.

Results

In total, twelve female *T. tropicanus* (6.8–207.8 g, 8–30 cm total body length) and seven male *T. tropicanus* (9.7–117.5 g, 11.8–25.2 cm) were collected. The total body length, weight and sex of collected *T. tropicanus*, bacterial results, and the presence of parasites observed histologically in this study are summarized in Table 1. Bacteria were cultured from two individuals; a

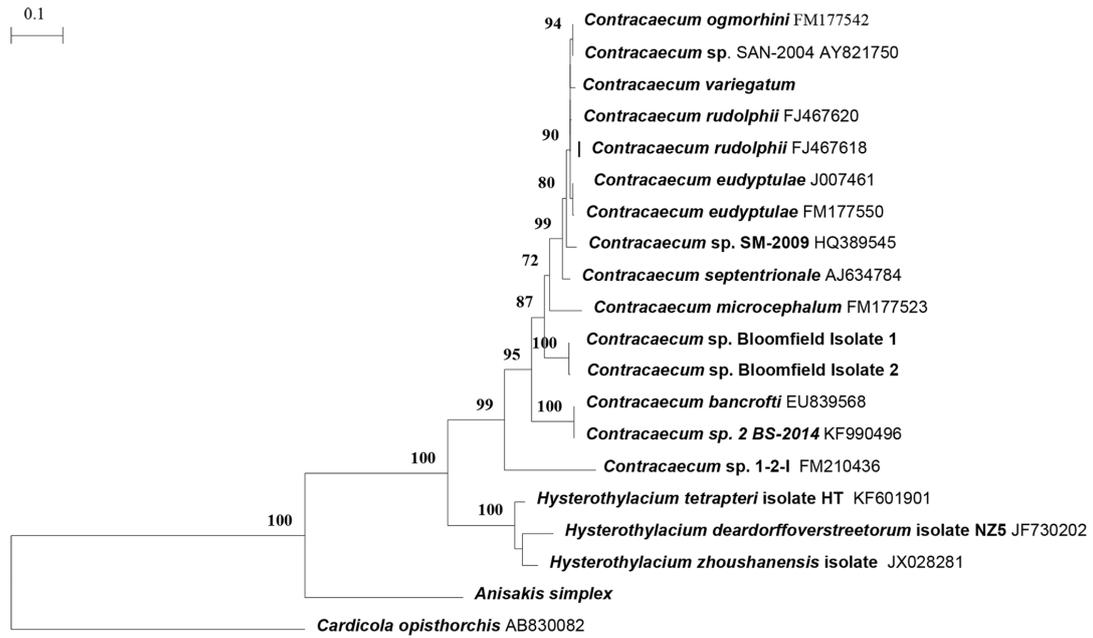


Figure 1. Evolutionary relationships of *Contracaecum* species inferred by distance analysis of ITS rRNA sequences (GenBank accession numbers listed after species name). Percentage support (>70%) from 1000 pseudoreplicates is indicated at the node.

Table 1. Sex, length, weight and key findings for each *T. tropicanus* sampled

Fish ID	Sex	Weight (g)	Total body length (cm)	Bacteria cultured	Nematodes (location observed)	Trematode metacercaria (location observed)	Monogeneans (location observed)	Epitheliocystis lesions
CBL-1	Female	39.2	18	<i>Aeromonas veronii</i>	-	-	Gills	-
CBL-2	Male	98.5	24.2	No growth	Intestinal mesentery	-	-	-
CBL-3	Female	35.2	17.5	No growth	-	Extra-ocular tissue	-	-
CBL-4	Female	35.0	19.0	No growth	-	Extra-ocular tissue	-	-
CBL-5	Male	32.9	15.5	No growth	Hepatic parenchyma	-	-	-
CBL-6	Male	32.3	16.7	No growth	Hepatic parenchyma	-	-	-
CBL-7	Male	117.5	25.2		-	-	Gills	-
CBL-8	Female	170.4	27.2	No growth	-	Extra-ocular tissue	Gills	-
CBL-9	Female	76.3	20.8	No growth	Peri-hepatic connective tissue, and intestinal lumen	-	Gills	-
CBL-10	Female	146.3	27.0	No growth	-	-	-	-
CBL-11	Female	96.5	27.8	No growth	Hepatic parenchyma	-	-	-
CBL-12	Female	207.8	30.0	No growth	-	-	-	-
CBL-13	Male	13	12.5	No growth	Intestinal mesenteric tissue, and hepatic parenchyma	-	-	-
CBL-14	Female	42.4	18.0	No growth	-	-	-	-
CBL-15	Male	9.7	11.8	No growth	-	Extra-ocular tissue	-	-
CBL-16	Male	57.7	21.0	No growth	Intestinal mesentery	-	-	Present
CBL-17	Female	14.5	13	No growth	Peri-hepatic connective tissue	-	-	-
CBL-18	Female	6.8	8	<i>Aeromonas veronii</i> , <i>Aeromonas jandaei</i> , <i>Bacillus Lactobacillus</i> - like organism	-	-	-	-
CBL-19	Female	41.3	18.0	No growth	Mesentery	-	-	-

light growth of *Aeromonas veronii*, *Aeromonas jandaei* and *Bacillus/Lactobacillus*-like organisms were cultured from one fish, and two colonies of *A. veronii* were cultured from a second fish. Histologically, trematode metacercariae were observed in the extra-ocular tissue of four fish (prevalence = 21.1%, 95% CI = 7.5-44.6%), associated with no apparent host response. A light unidentified monogenean infection was observed in the gills of four fish (prevalence = 21.1%, 95% CI = 7.5-44.6%), in association with mild multifocal hyperplasia, inflammation and necrosis of the tips of a small number of secondary gill lamellae. In one fish, three basophilic spherical cysts with eosinophilic capsules were present on one primary filament. These lesions are typical of epitheliocystis, and were associated with mild epithelial lifting and inflammation.

Nematodes were observed histologically in nine fish (prevalence = 47.4%, 95% CI = 25.7-68.8%). Nematodes encapsulated by fibrous tissue were observed within the hepatic parenchyma (Figures 2 and 3), in per-hepatic connective tissue and intestinal mesenteric tissue. Encapsulated nematodes were associated with an inflammatory cell infiltrate predominantly composed of lymphocytes, and few heterophils and eosinophilic granular cells. Nematodes were observed within the intestinal lumen of only one fish, and were not associated with an inflammatory response.

Sequence analysis of the ITS region of the rRNA gene was conducted on two isolates of these nematodes (GenBank accession number KM463760 and KM463761). These shared an identical sequence and fell within a clade containing species of *Contracaecum* on the phylogenetic tree (Figure 1). The isolates presented 93% similarity to *Contracaecum septentrionale* (Li et al. 2005) and 91% similarity to *Contracaecum bioccai* (D'Amelio et al., 2012).

No significant histological changes indicative of disease or poor health were observed in the muscle, eye, brain, heart, kidney, gastro-intestinal tract, spleen or gonads.

Discussion

T. tropicanus is a newly described species, reported only in coastal rivers of the Wet Tropics region of north-eastern Queensland (Welsh et al. 2014). This study provides the first report on the health of *T. tropicanus*, including the bacterial pathogens and parasites present in this species. Histopathology is considered the most useful initial test for assessment of fish health in Australia (Handler 2008). In this study, all *T. tropicanus* appeared healthy, however, lesions typical of epitheliocystis were observed histologically in one fish. This is the first report of epitheliocystis in *T. tropicanus*, and in any Australian freshwater catfish (Stride 2014). Two *T. tropicanus* individuals tested positive for *A. veronii* and *A. jandaei*, two bacterial species which have been reported as potentially serious pathogens of fishes (Esteve et al. 1993; Cai et al. 2012), and humans (Joseph et al. 1991). However, as there was no clinical or histopathological evidence of bacterial disease, it is likely the *Aeromonas* spp. are infections secondary to the stress of capture and low ambient temperatures or a result of contamination during sample collection.

Molecular and phylogenetic analysis suggest that the nematodes observed within the liver are a species of *Contracaecum*. (Ascaridida; Anisakidae). *Contracaecum* spp. have been reported from a wide range of native Australian fishes, including Black bream *Acanthopagrus butcheri*, King George whiting *Sillaginodes punctata*, Sea mullet *Mugil cephalus* and Yellow eyed mullet *Aldrichetta forsteri* (Lymbery et al. 2002), Common galaxias *Galaxias maculatus* (Chapman et al. 2006), Nightfish *Bostockia porosa*, and western minnow *Galaxias occidentalis* (Hassan 2008); however molecular identification was not attempted in these studies. Johnston and Mawson (1950) identified encysted larval *Contracaecum* sp. within the mesentery of *T. tropicanus* from the Murray River; these were identified as likely *C. spiculigrum* or *C. bancrofti*, however, molecular identification was not undertaken. It is likely that piscivorous birds, including the Australian pelican (Johnston and Mawson 1941), are the definitive



Figure 2. Nematode (T/S) encapsulated by fibrous tissue. This parasitic stage has an intensely eosinophilic cuticle, a digestive tract and groups of myotomes (H&E, 20x magnification). Image credit: E. Kelly.

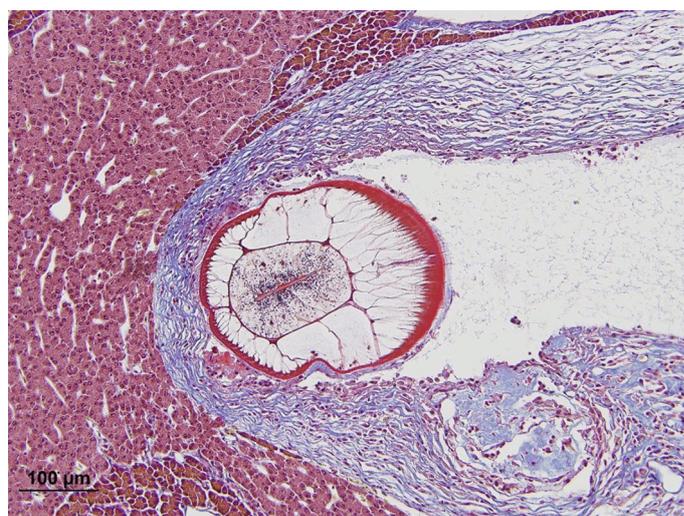


Figure 3. A nematode (T/S) surrounded by fibrous tissue (Martius scarlet blue, 20x magnification). Image credit: E. Kelly.

host for *Contracaecum* and assist in the spread of this parasite between river systems.

Although histological examination can only provide an overview of the tissues examined, our observations indicate a low prevalence of *Contracaecum* infection in the *T. tropicanus* sampled. The nematodes observed in this study appear to be co-existing with their catfish host without causing significant tissue damage. However, host parasite interactions can be labile, and any changes in host or environmental factors such as environmental degradation can change this healthy co-existence to one where disease is observed (Simkova *et al.* 2001). As in this study, the organ reported to be most commonly infected by *Contracaecum* spp. in fish is the liver, and heavy infections have been associated with grossly visible hepatic pathology (Lybery *et al.* 2002). Although invertebrates and fish are the most common intermediate hosts of *Contracaecum* spp., humans have been reported as accidental hosts (Lamps 2009). No *Contracaecum* larvae were observed in the muscle of sampled *T. tropicanus* in this study, suggesting a low risk of

zoonosis by consuming the muscle of caught *T. tropicanus*. A similar study by Lybery *et al.* (2002) also observed no *Contracaecum* larvae within the musculature of *A. butcheri*, *S. punctata*, *M. cephalus* and *A. forsteri*, and reported no post-mortem migration of larvae to the musculature.

This opportunistic sample provided a unique opportunity to study the general health and parasite fauna of a newly species of native freshwater catfish from a relatively undisturbed catchment. As the Bloomfield River experiences few anthropogenic disturbances, it is not surprising that the histology of *T. tropicanus* examined did not show significant tissue abnormalities indicative of disease. Mild epitheliocystis was observed in one fish, and this is the first report of epitheliocystis in this species. The *Aeromonas* spp. cultured in this study was likely related to the stress of capture. This is the first report of *Contracaecum* sp. in *T. tropicanus*, and based on initial molecular and phylogenetic analysis, is likely a new parasite species. These findings provide useful data for future assessments of fish health and ecosystem integrity in Australia.

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