IJP: Parasites and Wildlife 7 (2018) 12–17

Contents lists available at ScienceDirect

IJP: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Haematozoa of wild catfishes in northern Australia

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ARTICLE INFO

Keywords:
Trypanosome
Haemogregarine
Blood smear
Teleost

ABSTRACT

Very little is known about the diversity, prevalence, or pathogenicity of haematozoa in Australian freshwater fishes. Blood smears from 189 native catfishes, of six different species, from northern Australia were examined for haematozoa. Haematozoan infections were observed only in fishes from Queensland, at an overall prevalence of 0.191 (95% CI = 0.134–0.265). Intraerythrocytic haemogregarines were present in *Neoarius graeffei* from the Brisbane River at a prevalence of 0.35 (0.181–0.567). Trypanosomes were present in *Tandanus* species from four rivers, at prevalences ranging from 0.111 (0.020–0.330) to 1 (0.635–1), and in *N. graeffei* from one river in Queensland, at a prevalence of 0.063 (0.003–0.305). The haematozoans observed appeared to have little impact on their hosts. *Tandanus* spp. were significantly more likely to be infected with trypanosomes, suggesting a high parasite-host specificity. This is the first widespread survey of wild Australian freshwater catfishes for haematozoa, resulting in the first report of haemogregarines from Australian freshwater fish, and the first report of trypanosomes from *Neoarius graeffei* and *Tandanus tropicanus*.

1. Introduction

Parasitic haematozoa have been reported in a wide range of fish species worldwide. The most frequently reported haematozoa of fishes are kinetoplastids of the genera *Trypanosoma* and *Trypanoplasma* (*Cryptobia*), and apicomplexans belonging to genera of haemogregarines (Woo, 2006). Trypanosomes and haemogregarines are heteroxenous, and are believed to be transmitted to fish hosts by haematophagous vectors during feeding (Hamilton et al., 2005; Smit et al., 2006; Woo, 2006; Curtis et al., 2013), however a complete understanding of the life cycle of piscine haematozoa remains unknown.

Trypanosome species such as *Trypanosoma danilewskyi* and *T. murmanensis* develop into epimastigotes and metacyclic trypanosomes in the digestive system of their leech hosts (Qadri, 1962; Khan, 1976). Metacyclic trypanosomes accumulate in the proboscis of the leech, are presumably transmitted to their fish hosts during feeding (Woo, 2006), and once in a fish host, trypanosomes such as *T. danilewskyi* replicate as trypomastigotes in the blood (Woo, 1981). The life cycle of haemogregarines is also believed to be heteroxenous, whereby fishes are infected either through ingestion of an intermediate host, or via the introduction of sporozoites into the host through the bite of an infected vector (Davies, 1995).

Only two species of haematozoa have been recorded from Australian freshwater teleosts. Johnston and Cleland (1910) recorded *Trypanosoma bancrofti* in freshwater catfish *Tandanus tandanus* in Queensland, and *Trypanosoma anguillicola* in Australian marbled eel *Anguilla reinhardtii* from New South Wales and Queensland. Mackerras and Mackerras (1961) recorded *T. bancrofti* and *T. anguillicola* from the same host species. Although systematic parasite surveys of native fish species are increasing in Australia, most do not involve examination of blood samples, and therefore it is likely many haematozoan species have not been recorded (Adlard and O'Donoghue, 1998).

The effects of haematozoan infections on individual Australian freshwater fish or the health of fish populations are unknown. Parasites may influence host population dynamics by directly affecting host morbidity and mortality, modulating host growth and reproduction, and altering the likelihood of predation in the wild (Barber et al., 2000). Several studies in Australia, for example, suggest that haematozoa such as trypanosomes may be contributing to the decline of endangered terrestrial mammal species such as the woylie *Bettongia penicillata* (Botero et al., 2013; Thompson et al., 2014), and Gilbert's potoroo *Potorous gilbertii* (Austen et al., 2009) that are already threatened by wider ecosystem changes. Like many of Australia's mammals, native freshwater fishes in Australia are highly endemic, and are increasingly

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https://doi.org/10.1016/j.ijppaw.2017.12.002
Received 26 October 2017; Received in revised form 8 December 2017; Accepted 13 December 2017
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threatened by anthropogenic habitat alteration, including the introduction of invasive alien species, exotic disease emergence, and habitat destruction. As certain haemoparasite species are pathogenic to fishes (Ferguson and Roberts, 1975; Khan, 1985; MacLean and Davies, 1990; Woo, 2006), they represent a potential threat to wild populations already under pressure.

Ariid and plotosid catfishes represent a large component of total fish biomass in many northern Australian rivers (Bishop et al., 2001; Jardine et al., 2012), and silver cobbler *Neoarius midgleyorum* forms the basis of Western Australia's only freshwater finfish fishery. Eel-tailed catfish *Tandanus tandanus* is currently listed as Threatened in Victoria under the Flora and Fauna Guarantee Act 1988 (Department of Sustainability and Environment Victoria, 2005), and as Endangered in the Murray-Darling Basin in New South Wales (NSW) under the NSW Fisheries Management Act 1994 (Fisheries Scientific Committee, 2008). Freshwater catfishes are often host to a highly diverse range of tissue parasites (Lymbery et al., 2010), however, no widespread study on the hematozoa of catfishes has been undertaken in Australia. Here, we report on the haematozoa of catfishes sampled from freshwater systems in northern Australia, and investigate the effect of fish size and species on parasite prevalence.

2. Materials and methods

2.1. Sample collection and preparation

Native catfishes were sampled from 11 localities across Western Australia, Queensland and the Northern Territory, using a combination of fyke nets, handlines and electrofishing, between May 2014 and February 2015 (Table 1; Fig. 1). Fishes were euthanised using a prolonged anaesthetic bath of isoeugenol (AquiS, Lower Hutt, New Zealand), examined by eye for ectoparasites, and body weight and total body length were recorded. Blood was collected by caudal vertebral venepuncture, or following excision of the caudal peduncle in small fishes, as described by Kelly and Gibson-Kueh (2015), and major organs were collected and processed for histology as described by Kelly and Gibson-Kueh (2017). One air-dried blood smear per fish was fixed in methanol and stained with Wright-Giemsa (Kinetic, Caboolture, Queensland).

2.2. Microscopic evaluation

Blood smears were systematically scanned using 10x objective lens, followed by closer examination with 40x (high-dry) and 100x oil immersion objective lens (Stockham and Scott, 2008). A sample was considered uninfected if no haematozoa were observed after 15 min of scanning with the 100x oil immersion objective lens (Salkeld and Schwarzkopf, 2005). Histological sections of tissues from infected fishes were examined for the presence of tissue and blood borne parasite stages. Slides were examined on an Olympus BX41 laboratory microscope, and images taken on an Olympus BX51 system microscope, using an Olympus DP70 microscope digital camera and software (www.olympus.com).

2.3. Infection parameters

Prevalence was estimated separately for haemogregarine and trypanosome parasites (see Results), for each fish species in each locality, with 95% confidence intervals calculated assuming a binomial distribution, using the software Quantitative Parasitology 3.0 (Rózsa et al., 2000). Fisher exact tests were used to compare differences in prevalence between fish species or genera. Differences in length and weight between infected and uninfected fishes were tested using a non-parametric Wilcoxon test, with a normal approximation.

2.4. Morphometric analyses

Digital images were used to measure key morphological features of haemogregarines (Table 2), and trypanosomes (Table 3), as utilized by Smit et al. (2006) and Mackerras and Mackerras (1961) respectively, using Image J software (open source Java image processing program, available from http://imagej.net/Downloads; Schindelin et al., 2012). Trypanosomes were divided into two different morphological groups on the basis of one morphological trait (KN; see Results). Differences between these groups over all other morphological traits were tested using multivariate analysis of variance (MANOVA) and differences between groups for each trait were tested using one-way analyses of variance (ANOVA), with a Bonferroni correction to maintain an experiment-wide error rate of 0.05. All morphological data were log-transformed and the residuals from all analyses were normally distributed. Where the MANOVA showed a significant difference between groups, stepwise discriminant analysis was used to find the best combination of traits separating the groups. All statistical analyses were implemented in JMP®, Version 10.0 (SAS Institute Inc., Cary, NC).

3. Results and discussion

Blood smears from 189 catfishes, representing six species, were examined (Table 1). No haematozoa were observed in fishes from the

Table 1
Haematozoa present in catfishes by species and collection location.

Sampling location	Latitude (◦ S)	Longitude (◦ E)	Fish species collected (n)	Prevalence (95% CI)	
				Trypanosomes	Haemogregarines
Brisbane River	27·5447	152·7837	*Neoarius graeffei* (20)	0	0.350 (0.167–0.576)
Burnett River	25·2304	152·0116	*Neoarius graeffei* (16)	0.062 (0.003–0.305)	0
Barron River[a]	17·2611	145·5378	*Tandanus tandanus* (18)	0.111 (0.020–0.330)	0
Bloomfield River	15·9868	145·2882	*Tandanus tropicanus* (19)	0	0
Tully Catchment[a]	17·8818	145·8412	*Tandanus tropicanus* (18)	0.333 (0.156–0.586)	0
Palm Tree Creek (Pioneer River)	21·1540	148·7266	*T. tandanus* (3)	0	0
Mary River (site A)	26·0342	152·5106	*Neoarius graeffei* (10)	0	0
			Tandanus tandanus (8)	0.250 (0.046–0.635)	
Mary River (site B)	26·3319	152·7020	*Neosilurus hyrtlii* (1)	0	0
			Neoarius graeffei (1)	0	0
			Tandanus tandanus (8)	1 (0.635–1)	0
Goondaloo Creek (Ross River)	19·3232	146·7630	*Neosilurus hyrtlii* (1)	0	0
			Neosilurus ater (13)	0	
Ord River	15·7932	128·7177	*Neoarius graeffei* (11)	0	0
			Neoarius midgleyorum (13)	0	0
Rapid Creek	12·3955	130·8722	*Neosilurus hyrtlii* (29)	0	0

[a] At these sites, a number of locations were used to capture the required number of fishes and the coordinates refer to the modal locality.

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