Helminth parasitism of *Galaxias maculatus*(Jenyns 1842) in southwestern Australia

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Abstract

One cestode, *Ligula* sp. [possibly *Ligula intestinalis* (L.)], one trematode, *Diplostomum* sp., and two nematode parasitic worms, *Eustrongylides* sp. [possibly *Eustrongylides gadopsis* (Royal Society of South Australia, 64, 340)] and *Contracaecum* sp. are reported from *Galaxias maculatus* inhabiting a permanent freshwater lake and two intermittently flowing, saline rivers in southwestern Australia. Worms infecting fish are all larval; the definitive hosts are piscivorous waterfowl. *Ligula* sp. infected 12% of fish in the lake. Effects of infection include reduced lifespan, significant weight reduction of gonads of males and females and body weight of females. Infection reduces the proportion of males that attain spawning gonad stage more severely than it does in females. The prevalence and intensity of *Ligula* sp. infection were much less in the rivers. The infection of *Pseudogobius olorum* (Sauvage 1880) by this cestode is reported for the first time in Western Australia. Trematodes were much more benign in their effect on *G. maculatus*.

Keywords: *Ligula* sp.; plerocercoids; reduced lifespan; gonad and body weight loss

Introduction

The spotted minnow, *Galaxias maculatus*, is a small, scaleless, galaxiid with a widespread southern hemisphere, circumpolar distribution that includes Australia, New Zealand, South America and the Falkland, Lord Howe and Chatham Islands (Berra *et al.* 1996). In Western Australia, it occurs on the south coast between the Walpole River (34 °59′S, 116 °45′E) and the Thomas River (33 °50′S, 123 °00′E) with one west coast record from the Harvey River (33 °05′S, 115 °40′E) (Morgan et al. 2005).

Parasitism of freshwater fish in natural environments in Australia has not been the subject of extensive research. The most recent treatment of the subject is by Humphrey & Langdon (1986), which addressed mainly parasites of fish in aquaculture but also included two papers,Callinan (1986) and Harris (1986) pertinent to fish in natural environments. Beumer et al. (1983) list 15 species of protozoan, helminth and trematode parasites from five species of Australian *Galaxias* of which nine are recorded from *G. maculatus* but none from Western Australia.

Pollard (1974) examined cestode and nematode parasitism of *G. maculatus* from Lake Modewarre in southwest Victoria and reported the first record for the Australasian region of *Ligula intestinalis*. Pollard (1974) also reported parasitism by the larval stage of *Eustrongylides* sp. (possibly *Eustrongylides gadopsis*) in the visceral peritoneum. Morgan (2003) reported the first record of *L. intestinalis* from Western Australia from infected *Galaxias truttaceus* Valenciennes (1846) and *G. maculatus* from Moates Lake.

The aims of this study were to identify some parasites of *G. maculatus* in Western Australia and determine their effects on host fish including determination of which size/age classes are predominantly infected and how different habitats influence prevalence and severity of infection. In particular, previous suggestions that brackish-saline waters reduce the prevalence of cestode infection and that it is limited to smaller fish in some situations were examined.

Materials and Methods

Three sites, one a permanent freshwater lake, Moates Lake and two intermittently flowing, brackish-hypersaline rivers, the Jerdacuttup and Oldfield Rivers, were intensively sampled. A sample of 50+ fish was netted by 10 m seine net with a 3-mm mesh from each site monthly over an 18-month period as part of a wider study of *G. maculatus*.

Study sites

Moates Lake is a permanent freshwater lake located in Two Peoples' Bay Nature Reserve approximately 30 km east of Albany, Western Australia at 34 °58′S, 118 °07′E. Electrical conductivity (EC) of lake water ranged from 0.75 to 1.6 $mS \cdot cm^{-1}$ for the duration of this study.

The Jerdacuttup River site is a semi-permanent pool approximately 15 km east to northeast of Ravensthorpe, Western Australia at 33 °33′S, 120 °09′E. The EC range of river water was 11.0–63.0 $mS \cdot cm^{-1}$.

The Oldfield River site is a permanent pool at 33 °33′S, 120 °32.00′E, approximately 46 km east of Ravensthorpe, Western Australia. The EC range of river water was 28.3–94.2 $mS \cdot cm^{-1}$. EC data are from Chapman et al. (2006).

Laboratory procedures

Total and standard lengths (TL and SL) of fish were measured to the nearest millimetre and fish were wet weighed to 1 mg. Fish were previously preserved in 75% ethanol. Parasites were removed, counted and weighed to the nearest milligram concurrently with gonadal dissection and examination. Only parasites evident in the coelomic cavity or encysting internal organs were considered in this study. Parasites weighing <0.001 g were assigned an arbitrary weight of 0.0005 g. *Ligula* sp. were identified by the furrowed scolex dome with the furrow extending down both sides of the body. The body is also transversely annulated. Identification was assisted by reference to and comparison with the scanning electron microscope image in Morgan (2003). Prevalence of infection and relative mass

(% cestode weight = cestode weight/fish weight − cestode weight × 100) were recorded. Nematodes and trematodes were identified by RH at the Murdoch University veterinary parasitology laboratory with reference to the original descriptions in Johnston & Mawson (1940) and Smith & Hickman (1983). Nematodes were cleared for identification with lactophenol. Gonad stages are according to the criteria of Laevastu (1965)i.e., stage I – virgin, stage II – maturing virgin, stage III – developing, stage IV – developed, stage V – mature or gravid, stage VI – spawning and stage VII – spent. SL is the straight-line distance from the snout to the posterior end of the caudal peduncle. Gonadosomatic index (GSI) is given by the equation GSI = wet weight of gonad/wet weight of fish × 100. Fish designated 0+, 1+ are in their first and second years, respectively.

Results

Numbers of fish examined, with numbers infected by each parasite from each site are given in Table 1.

Cestode infections –Ligula sp. infection at Moates Lake

Ligula sp. was found within the coelomic cavity. With growth, they tightly envelop internal organs particularly gonads and intestine causing both internal distortion and external disfiguration. Infection prevalence was 12.4%. Forty-one per cent were females ranging from 40 to 76 mm TL. Males ranged from 36 to 61 mm TL. Between one and eight cestodes were present, the mean numbers of parasites per infected fish were 2.6 for males and 2.6 for females. The relative mass of parasite to host weight ranged from 0.19% to 114.62% (mean 28%) for males and from 0.45% to 116.90% (mean 24%) for females. Seasonal prevalence were summer 7.01%, autumn 19.41%, winter 10.44% and spring nil. Cestodes ranged in length and weight from 2.5 mm weighing 0.0005 g to 45 mm weighing 0.197 g.

Measurements of total length, fish weight, GSI and gonad weight are in Table 2. Fish weight is wet weight of fish less total weight of *Ligula*sp.

Although these data indicate that infected fish do have reduced gonad weight and GSIs, the differences are significant ($P < 0.05$) for gonad weight of both sexes but GSI only for males. Differences in total length and weight are significant for females only. Table 3 indicates percentages of infected and uninfected fish in different gonad stages. A small number of infected fish do reach gonadal stages V and VI. One stage VI female had one cestode with relative mass to body weight of 26.0%; three others at stage V had a mean number of 3.3 cestodes and a mean relative mass of 25.5% to body weight. Two males at stage VI had one cestode each with a mean relative mass of 16% to body weight; seven at stage V had a mean of 2.1 cestodes with a mean relative mass of 32.8% to body weight.

Table 2 indicates that infected fish occupy a reduced total length range within the range of uninfected fish. The age structure of infected fish was 81% and 19% for 0+ and 1+ fish, respectively, compared with 75%, 21%, 3.9% and 0.1% for 0+, 1+, 2+ and 3+ fish, respectively, for uninfected fish. Although this suggests that only younger fish are infected, the difference is not significant ($\chi^2 = 4.45$, $P = 0.1081$), but the strength of the statistical test is limited by very low expected values.

A sample of 45 *Pseudogobius olorum* (22–44 mm TL) from Moates Lake, which were examined for *Ligula* sp. from January, February, April and May had nil infection.

Cestode infections –Ligula sp. infection at southeast rivers

Ligula sp. prevalence at the Oldfield River was 0.38% with between one and four cestodes per fish. All were females ranging from 64 to 132 mm TL. The larger fish was the largest fish collected from this river; it also had a nematode infection (see below). Relative mass to body weight ranged from 1.63% to 7.87%.

Seven of 13 *P. olorum* (20–40 mm TL) collected in September from the Oldfield River were infected with between one and three cestodes. This is the first record of *Ligula* sp. infection of *P. olorum* in Western Australia. There was one infected *P. olorum* from by-catch fish from the Jerdacuttup River; other infected fish, of hundreds released, may have been overlooked.

Trematode infections –'black spot disease' infection of fish from Moates Lake

'Black spot disease' is evident as small, dark, raised lesions on the skin. On removal of the lesion, metacercarial cysts of the strigeoid trematode, *Diplostomum* sp. (probably *Diplostomum galaxiae*) are encysted in the muscle. Prevalence of *Diplostomum* sp. was 11.78%. TL of infected fish ranged from 46 to 101 mm with both sexes approximately equally affected. Number of metacercariae per fish ranged from one to eight with a mean of 2.2 per infected fish. Seasonal prevalence were summer 1.72%, autumn 26.01%, winter 21.49% and spring nil. The percentage of infected fish varied with age, with 30.4%, 63.6%, 4.0% and 2.0% of 0+, 1+, 2+ and 3+ fish infected, respectively. Only two fish had concurrent infections of *Ligula* sp. and *Diplostomum* sp. Fifteen infected males and five females were at gonad stage VI, therefore it seems unlikely that infection inhibits reproductive success.

Nematode infections – larval nematode infection of fish from Moates Lake

A larval nematode similar to that described by Johnston & Mawson (1940) as *E. gadopsis* was present at a prevalence of 1.93%. The nematode was encysted in the inner body wall in all, but one case where the worm was encysted in the ovary. Sixty-nine per cent of infected fish were females ranging from 48 to 107 mm TL. Infected males ranged from 39 to 65 mm TL Number of nematodes ranged from one to four, mean number of parasites per infected fish was 1.6. Relative masses ranged from 0.06% to 4.35% with a mean of 1.16%. Nematodes were only found over the months February–July. Two females were at gonad stage VI with nematode relative masses of 1.07% and 4.35% and one at stage VII; therefore it seems unlikely that here nematode infection inhibits reproductive success. Larval*Eustrongylides* infecting *G. maculatus* here were all relatively small worms; the largest was 55 mm long and weighed 0.052 g

Nematode infections – larval nematode infection of fish from southeast rivers

Prevalence of *Contracaecum* sp. from the Jerdacuttup River was 0.24% with nematodes encysted in the inner body wall. Both fish were males, 35 and 56 mm TL with one nematode each. Relative masses were 0.23% and 0.29%.

Prevalence of *Eustrongylides* sp. from the Oldfield River was 0.13% with nematodes encysted in the inner body wall. One was a female, at 132 mm TL; it was the largest fish collected from this river; it also had a *Ligula* sp. infection.

Discussion

All the parasitic worms found in *G. maculatus* in this study were larval stages of worms, which reach the adult stage in piscivorous birds. Although *L. intestinalis* has twice been previously recorded in Australia (see above), we are unable to categorically assign our larval cestodes to this taxon because we were unable to complete the plerocercoids life cycle experimentally. The life cycle of *Ligula* sp. involves the voiding of eggs into the water with the faeces of piscivorous birds and their subsequent ingestion by copepods, the first intermediate host, which in turn are eaten by fish, the second intermediate host, where they develop into plerocercoid larvae (Pollard 1974). The larvae may survive for 12–14 months in their fish hosts. When fish are eaten by birds, the elevated body temperature stimulates gonadal development and the eggs are produced within 2 days. The adults usually only live in their bird hosts for up to 5 days (Dubinina 1954, cited by Pollard 1974). Fish are also the second intermediate hosts for *Diplostomum* sp., and are infected by external penetration of the skin by the cercariae released from an aquatic snail, the first intermediate host (Smith & Hickman 1983).

Cestode infections

As in the Pollard (1974) study at Lake Modewarre, only adult fish at Moates Lake are visibly infected with *Ligula* sp. with larger fish in the 0+ and 1+ age classes having larger and more larvae than smaller fish. The mean prevalence at Lake Modewarre was 46% that is far greater than 12.4% at Moates Lake. However, the mean number of parasites per infected fish was only 1.5 compared with 2.6 at Moates Lake.

Infection with *Ligula* sp. appeared to be restricted to the 0+ and 1+ age classes of fish, although low sample size of older fish precluded statistical significance. A lack of infection of older fish could be

explained by higher mortality of infected fish, perhaps by increased avian predation. Williams & Jones (1994) suggested that *L. intestinalis* may increase morbidity by causing a decrease in the number of muscle fibres, and by reducing amino acid and glucose levels.

Data not shown (Chapman 2003) indicate that fish of all age classes fed on the copepod intermediate hosts when the latter were abundant in the lake, so it is unlikely that feeding preferences alone explain the absence of infection in older fish.

Although seasonal prevalence indicated nil infection in spring (see above), it was likely that initial infection was in spring but not detected because of small size of cestodes, as calanoid copepods, the first intermediate host, were super abundant in spring when compared with other seasons (Chapman 2003). Infection was first detected in summer and was maximal in autumn with nearly 20% of fish infected.

The results of this study are in general agreement with those of Pollard (1974) in that cestode infection was found to reduce body condition and gonadal maturation. Although some infected fish from Moates Lake achieve reproductive condition as indicated by attainment of gonad stage V or VI, this does not necessarily mean that they successfully spawned. The survival chances of *L. intestinalis*-infected fish are reduced (Nikolsky 1963, cited by Pollard 1974) because of curtailed mobility due to disfiguration, and they may not reach a spawning area or may be taken as prey by piscivorous birds while doing so. The present study does not provide any evidence of cestode-induced host gigantism for Moates Lake; however, the very few infected fish from the river sites were the largest fish collected. Loot et al. (2002) reported that parasite-induced host gigantism was differentially present in different populations of roach *Rutilis rutilus* (L.) in southwestern France.

Pollard (1974) suggested that the agents of introduction of *L. intestinalis* from the northern hemisphere to Australia could have been redfin perch or brown trout from Europe or rainbow trout from North America. These were all present in Lake Modewarre and have been recorded as *L. intestinalis* hosts in the northern hemisphere. The arrival of adult *L. intestinalis* via migratory birds is most unlikely for two reasons: the adult stage is short lived (<5 days) and transequatorial migrant

birds entering Australia do not feed on freshwater fish. However, there are numerous piscivorous birds including grebes, cormorants, herons, egrets, pelicans and kingfishers (see Barker & Vestjens 1989), which are nomadic or locally very mobile and could act as definitive hosts once the parasite was introduced.

Morgan (2003) considered it likely that the introduction of *L. intestinalis* to Moates Lake may have been through the release of brown or rainbow trout in the 1950s or 1960s or by a local avian host. Introduction to southeast rivers, where no infected fish are, or have been present, could be by white-faced heron, *Egretta novaehollandiae* (Latham 1790); little pied cormorant, *Phalacrocorax melanoleucos*(Vieillot 1817) or hoary-headed grebe, *Poliocephalus poliocephalus* (Jardine & Selby 1827) all of which are piscivorous, locally common and the former two are known galaxiid predators (Barker & Vestjens 1989). There is also abundant circumstantial evidence to implicate hoary-headed grebe as a major galaxiid predator (Chapman 2003).

Variations in prevalence of *L. intestinalis* between different localities may be due to factors such as abundance of definitive and intermediate hosts, water temperature and salinity as well as age achieved by host fish. Dubinina (1954) (cited by Pollard 1974) reported that relatively high water temperature favours *L. intestinalis* and brackish-saline water disfavours it. Brackish-saline water as well as the scarcity of calanoid copepods might account for the low prevalence in southeast rivers.

Trematode infections

'Black spot disease' caused by *Diplostomum* spp. cysts had not been recorded in Australia until Smith & Hickman (1983) described a new species of trematode, *D. galaxiae* from *Galaxias auratus* (Johnston 1883) from Lake Crescent in Tasmania. The host for the adult fluke is a piscivorous bird, the white-faced heron that was present at Moates Lake (Jaensch et al. 1988) and was also common at the sampling sites on the Oldfield and Jerdacuttup Rivers where, however, infected fish were not recorded.

Prevalence of both *L. intestinalis* and *Diplostomum* sp. at Moates Lake showed a similar seasonal trend with low numbers in summer, maximum numbers in autumn that declined in winter to none in

spring. *Galaxias maculatus* here has a short lifespan, with the majority of fish living only up to approximately 1 year. Additionally, most fish in autumn are adults and most fish in spring are small juveniles (Chapman et al. 2006). Therefore, the seasonal prevalence of the larval worms in *G. maculatus* is probably primarily determined by the annual growth cycle of the fish.

Nematode infections

The life cycles and distribution of *Eustrongylides* and *Contracaecum* spp. are poorly understood; definitive hosts are Ardeiform birds (i.e., herons and egrets) with numerous fish species reported as intermediate hosts.

The prevalence of larval *Eustrongylides* sp. was much less at Moates Lake (1.93%) than it is at Lake Modewarre where 82% of fish were infected (Pollard 1974). Fish at the latter site had between 1 and 44 larval worms with a mean of 5.4 per infected fish (cf. 1.6 at Moates Lake).

Summary

1. One cestode, *Ligula* sp.; one trematode, *Diplostomum* sp.; and two nematode parasitic worms, *Eustrongylides* sp. and*Contracaecum* sp. are reported infecting *G. maculatus* in one freshwater lake and two saline rivers in southwestern Australia. The cestode caused gross body distortion, reduced lifespan, weight loss of male and female gonads and somatic weight loss of females. The trematode and nematodes are less malignant in their effects on *G. maculatus*.
2. The prevalence and intensity of cestode infection were significantly less in the rivers possibly because of their salinity. There was limited evidence for parasite-induced gigantism in one river but not in the lake.
3. In the lake, only smaller fish were infected by the cestode, suggesting that there is either increased mortality of infected fish or increased susceptibility to predation by piscivorous birds.

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Table 1. Numbers of *Galaxias maculatus* examined for parasitic infection from different sites with parasite prevalence and probability of parasites being equally distributed between sites.

	Moates Lake	**Jerdacuttup River**	**Oldfield River**	$\chi 2$ (P)
No. of *Galaxias maculatus* examined	831	825	799	
No. of *Ligula* sp.-infected fish	103	0	3	196·3 (<0·005)
No. of *Diplostomum* sp.-infected fish	98	0	0	194·0 (<0·005)
No. of *Eustrongylides* sp.-infected fish	16	0	1	26·9 (<0·005)
No. of *Contracaecum* sp.-infected fish	0	2	0	NA

Table 2. Measurements (mean ± SE) and range for *Ligula* sp.-infected and -uninfected *Galaxias maculatus* at Moates Lake.

Measurement	**Infected fish**	**Uninfected fish**
Total length (mm)		
Males	50.2 ± 0.79 (N = 58)	52.5 ± 1.46 (N = 286)
Females	53.2 ± 1.33 (N = 40)	60.5 ± 2.53 (N = 426)
Total length range (mm)		
Males	36–61	30–76
Females	40–76	30–107
Fish weight (g)		
Males	0.42 ± 0.01	0.56 ± 0.05
Females	0.52 ± 0.04	0.93 ± 0.13
Gonad weight (g)		
Males	0.011 ± 0.003	0.035 ± 0.007
Females	0.012 ± 0.005	0.036 ± 0.014
Gonadosomatic index (%)		
Males	1.81 ± 0.39	6.22 ± 0.75
Females	1.74 ± 0.77	2.80 ± 0.81

Table 3. Percentage of *Galaxias maculatus* in each gonad stage for *Ligula* sp.-infected and -uninfected fish at Moates Lake.

	Gonad stage						
	I	II	III	IV	V	VI	VII
Infected males (N = 58)	43.3	23.2	10.2	8.5	11.4	3.4	0
Infected females (N = 40)	26.8	61.1	0	0	7.3	2.4	2.4
Uninfected males (N = 286)	42	12.9	6.3	7.3	16.1	15	0.4
Uninfected females (N = 426)	23.5	31.2	17.4	11.5	7.3	3.5	5.6