Advances in life science research

Trematode parasite infection in the eyes of the freshwater fish *Haludaria fasciata* (Jerdon, 1849) from the South Western Ghats

Jithila P J[a] | Nisha P V[a] | Prasadan PK[a] | Abaunza P[b]

[a]Ecological Parasitology and Tropical Biodiversity Laboratory, Department of Zoology, Kannur University, Mananthavady Campus, Wayanad- 670645, Kerala, India, Corresponding author email: prasadanpk@kannuruniv.ac.in
[b]Instituto Espanol de Oceanografia (IEO)C.O. de Santander Promontorio San Martin s/n39004, Santander, Cantabria, Spain
Phone:+919847803136

ABSTRACT

Fishes act as intermediate hosts to diverse bird trematode parasites and this may significantly affect fish health. Eye infection, particularly of the lens, with strigeid and diplostomid metacercariae may result in blindness and increasing susceptibility to predation. Live specimens of freshwater fish *Haludaria fasciata* were collected from water bodies of Wayanad. The fishes were observed for behavioural studies and examined for metacercariae. The parasitological descriptors of infection were measured. Two species of digenetic trematode metacercariae, *Diplostomid metacercaria* and *Tetracotyle wayanadensis* were recovered from eyes of the freshwater fish *H. fasciata*. The parasitological descriptors of infection of *Diplostomid metacercaria* and *T. wayanadensis* were noted. The infected lens showed deformations in its gross morphology and theinfected fishes showed slow movements. Infection of larvae in the eyes of fishes may results in the formation of cataract leading to blindness which leads to impairments in the foraging efficiency and competitive ability. This eventually increases the chance of predation by the final host and promotes trophic level transfer of developing stages of parasites.

KEYWORDS

Diplostomid metacercaria; Tetracotyle wayanadensis; Western Ghats; Haludaria fasciata; digenetic trematodes

Introduction

Fishes are hosts to taxonomically diverse helminth parasites and may significantly affect fish health (Woo 2006; Seppanen et al. 2009). The parasites and pathogens are important causes for economic loss in aquaculture, in terms of reduced fish growth and increased mortality (Menzies et al. 2002; Hakalahti and Valtonen 2003). Trematodes of the family Diplostomidae Poirier, 1886 and Strigeidae Railliet, 1919 use freshwater fishes as their second intermediate hosts and outbreaks of these parasites were recorded from many hatcheries (Voutilainen et al. 2009; Kuukka-Anttila et al. 2010). Eye infections, particularly of the lens, with metacercariae of strigeids have been reported from many parts of the globe. The first record of Diplostomiasis came from Von Nordmann (1832) and numerous publications have been added to that study (Ferguson and Hayford 1941; Hoffmann 1960; Shariff et al. 1980; ChappelI et al. 1994) later. It has been suggested that parasites are protected from host immune responses in the lens, which lacks blood circulation (Chappell et al. 1994). Blindness was reported in such infections (Rushton 1938; Ferguson and Hayford l941; Shariff et al. 1980) which was followed by significant loss in bodyweight (Palmer 1939), increased susceptibility to predation (Seppala et al. 2004) and ultimate death by starvation (Dennis 1973).

Although reports on many studies on the morphology of diplostomids (Chakrabarti 1968; Pandey 1968; Chakrabarti and Baugh 1973; Agrawal and Khan 1982; Chaudhary et al. 2017a; Chaudhary et al. 20I7b) and strigeids (Rai and Pantle 1969; Baugh and Chakrabarti 1977; Pandey and Pandey 2000; Sheena and Janardanan 2008; Vankara et al. 2011) of freshwater fishes of India are available, their impacts on fish health and behaviour are scanty.

The Western Ghats is rich in freshwater fish diversity and endemism (Dahanukar et al. 2004). The freshwater fish *Haludaria fasciata* (Jerdon, 1849) is endemic to southern India and it contributes to the thriving aquarium industry of the Western Ghats regions (Raghaven et al. 2013; Chakraborty et al. 2017). This study deals with two metacercaria larvae of digenetic trematodes, *Diplostomid metacercaria* and metacercaria of *Tetracotyle wayanadensis* Jithila and Prasadan, 2018, infecting eyes of the freshwater fish *H fasciata* in the Western Ghats, Wayanad region with their prevalence, intensity and mean abundance of infection estimated.

Materials and methods

Collection and examination of fishes

Live specimens of *H. fasciata* collected from small rivulets in the Wayanad District, Kerala, India using sweep net were transferred to the Ecological Parasitological laboratory. The fishes were maintained in aquarium or cement cisterns and fed occasionally with fish meal. To study the behavioural changes the fishes, were observed continuously for two hours prior to dissection.

Examination of parasites

The sacrificed fishes were examined for parasites under a Labomed (Luxeo 4Z) stereozoom microscope and the metceracariae collected from the eyes were observed under a phase contrast research microscope (Nikon ECLIPSE Ni-U) with or without vital staining. For permanent preparation, parasites fixed in 5% formalin were stained with acetocarmine, dehydrated through ascending grades of alcohol, cleared in methyl benzoate and mounted in DPX (Cantwell 1981). Prevalence, mean intensity and mean abundance of infection were measured (Bush et al. 1997). Photographs and measurements were taken with the Nikon Y-TV55 camera and the Nikon NIS Elements Imaging Software attached to Nikon ECLIPSE Ni-U research microscope.

Results and discussion

In the present study *Diplostomid metacercaria* and metacercaria of *T. wayanadensis* (Figure I) were recovered from the eyes of the freshwater fish *H. fasciata.*

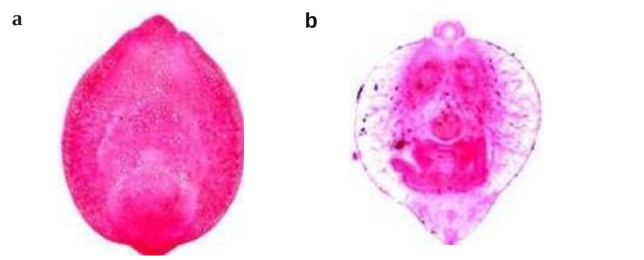
Diplostomid metacercaria, Taxonomic description:

Metacercariae without cysts were found freely moving in the eye. Aspinose body, oval, small, with pointed anterior and posterior ends and 838.45 - 915.35 x 625.89 - 654.60 (876.90 x 640.25) in size; hind body small and not well differentiated. Oral sucker round, smaller than ventral sucker and terminal in position having a size 39.77 -42.62 x 41.08-44.25 (41.20 x 42.67). Ventral sucker post equatorial, round to oval, 86.69-101.97 x 89.47 - 102.35 (94. 33 x 95.91) in size. The distance between oral sucker and ventral sucker 413.01 - 469.87 (441.97). Two accessory suckers present, one on either side of oral sucker. The distance between ventral sucker and posterior end of body 289.30 - 304.95 (297.16). Well developed hold fast organ present between the ventral sucker and the posterior end of caeca having 97.56- 139.97 x 1I 8.34 - I48.90 (118.76 x 133.62) size. The hold fast organ located 553.04- 649. 06 (601.05) distance away from anterior end. Mouth leads to prepharynx. Muscular pharynx and small esophagus present. Intestinal caeca extending up to posterior end of body, with maximum width at posterior end, 625.87 -674.81x 43.41 - 60.92 (650.34 x 52.17) in size. Excretory pore at the posterior end.

Tetracotyle wayanadensis Jithila and Prasadan 2018,
Taxonomic description:

Cyst oval, single-layered, thin, transparent, devoid of pigments. Body aspinose, broadly oval, divided into fore- and hind-bodies. Fore-body large, measured 687.10-987.24 x 670.20 - 902.71 (793.11 x 755.72) and hind body measured 186.95-405.62 x 236.22-407.78 (258.60x 301.95); length to width ratio of body is 1.38:1. Oral sucker terminal, round to ovoid, 67.55 - 104.82 x 55.18 - 184.48 (82.66 x 100.41). Ventral sucker ovoid, pre-equatorial in the forebody 84.17 - 128.36 x 105.45 - 133.54 (1l l.12x 119.90). Two prominent accessory suckers, one on either side of oral sucker, 212. 43- 337.94 x 100.73 -171.79 (280.39 x 135.01). Hold fast organ 168.00 - 295.48 x 324.55 - 445.98 (240.89 x 396.47), close to ventral sucker. Hold fast gland absent or not discernible. Prepharynx absent. Round to oval pharynx leads to esophagus. Intestinal caeca 552.79- 967.42 (723.34) extending up to posterior end of the body, width increases posterior to holdfast organ. Gonads represented by two oval, tandem masses, posterior to hold fast organ 20.00 - 50.17 x 41.44- 143.48 (35.93 x 96.05). Excretory bladder at posterior end of body; excretory pore terminal.

The sites of infection of *Diplostomid metacercaria* and *T. wayanadensis* were lens and vitreous humor of eyes respectively. *T. wayanadensis* was more prevalent while the intensity of infection was higher with *Diplostomid metacercaria* infection (Table I). Both metacercariae were in the excysted form agreeing Ginetsinskaya (1968), who pointed out that metacercariae inhabiting the eyes and the brain do not form cysts as they are not exposed to host secretions.



.Figure 1. a. *Diplostomid metacercaria and b. Tetracotyle wayanadensis* Jithila and Prasadan, 2018

Table 1. Parasitological descriptors of metacercarial infection in *H. fasciata*

Meatcercariae	Prevalence of irtfection (%)	Mean intensity of infection	Mean abundance of infection
Diplostomid metacercaria	1.59 (3/189)	3.33	0.05
Tetracotyle wayanadensis	5.29(10/189	2.60	0.14

According to Chappell (1995) *Diplostomid sp.* infection in the eyes of host fish is associated with the impairment of vision that leads to exophthalmoses and cataracts in the eyes. Similarly, according to Padro'sa et al. (2018) *Diplostomum* sp. infection in fish eyes can cause retinal lesions leading to visual impairments. In the present study, larvae of *Diplostomid metacercaria* found infected in the lens of *H. fasciata* were not having any cyst. In the present study unencysted *Diplostomid metacercariae* found in the lens (Figure 2) showed vigorous movements and also the infected lens showed deformations in its gross morphology. In a previous study Ashton et al. (1969) reported the survival of *Diplostomum spathaceum* in the lens for many months and even in dead fishes. Here also the parasites use lens as its protective covering.

Figure 2. Eye lensof H. fasciata. Diplostomid metacercaria inside the eye lens (arrow)

Data on three instances of mixed infection of these two parasites in the host were also noted during the present study. According to Ashton et al. (1969) the infection of *Diplostomum spathaceum* larvae in the fish results in the formation of cataract leading to blindness. Their study also pointed out that lens capsule thickening and associated epithelial cells proliferation was discernible. Same result was reported by Shariff et al. (1980) in the eyes of rainbow trout Salmo gairdneri infected with diplostomid metacercariae. I n the present study the deformation of lens was evident and this may result in trematode-induced blindness in fish.

Although parasite-induced behavioural changes are very common, the actual causes of such changes are often difficult to find out; adaptive manipulation by the parasite may be the cause, or adaptive responses by host, or side-effects of infection which are non-adaptive (Rosenkranz et al. 2018). According to Munoz et al. (2017) and Munoz et al. (2019) T. clavata infection in the eyes of fish impairs the competitive ability and foraging efficiency of the infected fish and they require more time for foraging to get similar food intake as of conspecifics which are non-infected. In the present study the infected fishes showed sluggish movements and poor intake of food when compared to their non-infected conspecifics. The study of Voutilainen et al. (2008) revealed that the *Diplostomum spathaceum* - induced cataract-bearing fish spends more time in foraging. This parasite-induced ethological changes in the fish may increase the predation risk and potentially enhance the transmission success to the final host.

Conclusion

Fishes serve as hosts to a wide range of taxonomically diverse parasites, among them the digenetic trematodes are the most important ones. Two metacercariae infecting the eyes of *H. fasciata* were found in the present study. The infected fishes showed sluggish movements and poor feeding activities. Parasites are dependent on the host communities that they live in, thus the presence of parasites in fishes may be useful indicators of fish health and aquatic health of the region.

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Conflict of interest

The authors declare that there is no conflict among them.

References

Agrawal N, Khan S (1982) A new Diplostomulum Brandes, 1892 metacercaria from the fish Heteropneutes fossilis. Indian J Parasitol 6:81-83

Ashton N, Brown N, Easty D (1969) Trematode cataract in fresh water fish. J Small Animal Practice 10:471 - 478. https://doi.org/10.111J/j.1748-5827.1969.tb04058.x

Baugh SC, Chakrabarti KK (1977) On a triple infection of strigeid metacercaria in an Indian freshwater fish Xenentodon cancila. Parasitol 18:84-94

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. Revisited. J Parasitol 83(4):575-583. https://doi.org/10.2307/3284227

Cantwell GE (1981): Methods for invertebrates. In: Clark G (ed) Staining procedures. Baltimore, Williams and Wilkins, pp. 255-280

Chakrabarti KK (1968) A new strigeid metacercaria Diplostomulum cerebralis n.sp. from Indian freshwater fish. Zoologischer Anzeiger 181:307-312

Chakrabarti KK, Baugh SC (1973) Studies on some strigeid metacercariae of the larval genus *Diplostomulum* Brandes, 1892 from Indian freshwater fishes. Rev lber Parasitol 33:107-125

Chakraborty A, Rumki S, Koushik G (2017) An inventory of endemic fish species in India with notes on state-wise distribution and conservation measures. Int J Fish Aquatic Studies 5(l):53-264

Chaudhary A, Gupta S, Verma C, Tripathi R, Singh HS (2017a) Morphological and molecular characterization of metacercaria of *Tylodelphys* (Digenea: Diplostomidae) from the piscine host, *Myslus tengara* from India. J Parasitol 103(5):565-573. https://doi.org/10.1645/16-52

Chaudhary A, Tripathi R, Gupta S, Singh HS (2017b) First report on molecular evidence of Tylodelphys cerebralis (Diplostomulum cerebralis) Chakrabarti, 1968 (Digenea: Diplostomidae) from

Chappell LH (1995) The biology of diplostomatid eye flukes of fishes. J Helminthol 69:97-101. https://doi.org/ I0.1017/S0022149XOOO13961

Chappell LH, Hardie LJ, Secombes CJ (1994) Diplostomiasis: the disease and host-parasite interactions. In: Pike AW, Lewis JW (eds) Parasitic diseases of fish. Samara, Dyfed, pp. 59-86

Dahanukar N, Raut R, Bhat A (2004) Distribution, endemism and threat status of freshwater fishes in the Western Ghats of India. J Biogeograpb 31:123-136

Dennis EA (l973) Development of the metacercaria and adhesive organ of *Mesostephanus yedere,* Dennis & Penner, 1971 (Trematoda: Cyathocotylidae) and their effects on the host tissue. J Helminthol 47:61-71. https://doi.org/10.1017/s0022149x00023749

Ferguson MS, Hayford RA(1941) The life history and control of an

eye fluke. Progressive Fish Culturist 54:1-13

Ginetsinskaya TA (I 968) Trematodes, their lifecycles, biology and evolution. Leningrad, USSR: Nouka

Hakalahti T, Valtonen ET (2003) Population structure and recruitment of the ectoparasite Argulus coregoni thorell (Crustacea: Branchiura) on a fish farm. Parasitol 127:79-85. https://doi.org/10.10l 7/S0031l 82003003l96

Hoffman GL (1960) Synopsis ofstrigeoidea (trematoda) of fishes and their life cycles. U.S. Fish and Wildlife Service, Washington, pp.439-466

Kuukka-Anttila H, Peuhkuri N, Kolari I, Paananen T, Kause A (2010) Quantitative genetic architecture of parasite-induced cataract in rainbow trout, Oncorhynchus mykiss. Heredity 104:20-27. https://doi.org/10.1038/hdy.2009.123

Menzies FD, Crockford T, Breck 0, Midtlyng PJ (2002) Estimation of direct costs associated with cataracts in farmed Atlantic salmon (Salmo salar). Bull Eur Assoc Fish Pathol 22:27-32

Munoz JCV, Staaks G, KnopfK (2017) The eye fluke Tylodelphys clavata affects prey detection and intraspecific competition of European perch (Perea fluviatiLis). Parasitol Res 116:2561-2567. https://doi.org/10.1007/s00436-017-5564-I

Munoz JCV, Bierbach D, Knopf K (2019) Eye fluke (Tylodelphys clavata) infection impairs visual ability and hampers foraging success in European perch. Parasitol Res. https://doi.org/10.1007/s00436-019-06389-5

Padrosa F, Knudsen R, Blasco-Costa I (2018) Histopathological characterisation of retinal lesions associated to Diplostomum species (Platyhelminthes: Trematoda) infection in polymorphic Arctic charr Salvelinus alpinus. Parasites and Wildlife 68-74. https://doi.org/10.1016/j.ijppaw.2018.01.007

Pandey KC (1968) Studies on freshwater fishes ofTndia III. On two new species of Diplostomulum Brandes, 1892. Proc Indian Acad Sci 72:167-170

Pandey KC, Pandey N (2000) Studies on two new stigeid metacercariae from Xenetodon cancila (Ham.). Indian J Helminthol 17:29-32

Raghaven R, Dahanukar N, Tlusty MF, Rhyne AL, Kumar KK, Molur S, Rosser AM (2013) Uncovering an obscure trade: Threatened freshwater fishes and the aquarium pet markets. Biol Conserv 164:158-169. http://dx.doi.org/LO.I016/j.biocon.2013.04.019

Rai P, Pande BP (1969) On the morphology and pathogenic significance of the strigeoid metacercariae in some Indian freshwater fishes, 1. Tetracotyle and Neascus. Indian J Anim Sci 39:355-359

Rosenkranz M, Poulin R, Selbach C (2018) Behavioural impacts of trematodes on their snail host: Species specific effects or generalised response?. Ethology 124:790-795. https://doi.org/10.ll11/etb.12808

Rushton W (1938) Blindness in freshwater fish. Nature 141:289. https://doi.org/10.1038/l 4l 289a0

Seppala 0, Karvonen A, Valtonen ET (2004) Parasite induced change in host behaviour and susceptibility to predation in an eye fluke-fish interaction. Anim behav 68:257-263. https://doi.org/10.10l6/j.anbehav.2003.10.021

Seppanen E, Kuukk.a H, Voutilainen A, Huuskonen H, Peuhkuri N (2009) Metabolic depression and spleen and liver enlargement in juvenile Arctic charr Salvelinus alpinus exposed to chronic parasite infection. J Fish Biol 74:553-561. https://doi.org/10.l Il l/j.1095-8649.2008.02144.x

ShariffM, Richards RH,Sommerville C (1980) The histopathology of acute and chronic infections of rainbow trout Salmo gairdoeri Richardson with eye flukes, Diplostomum spp. J Fish Dis 3:455-465. https://doi.org/l 0.1111/j.1365-276 l.1980.tb00432.x

Sheena P, Janardanan KP (2008) Six species of metacercaria (Digenea: Trematoda) from Etroplus maculates (Bloch) in the Chaliyar river ofKerala, India. Uttar Pradesh J Zoology 28:43-49

Vankara AP, Mani G, Vijayalakshrni C (2011) A report on various digenetic rnetacercariae from the freshwater fishes of River Godavari, Rajahmundry. J Parasit Dis 35(2):177-185. https://doi.org/10.1007/sl 2639-011-0041-8

Von Nordmann A (1832) Mikrographische Beitrasse zur Naturgeschichte der Wirbellosan. Tiere 1. Heft, Berlin
Voutilainen A, Figueiredo K, Huuskonen H (2008) Effects of the eye fluke Diplostomum spathaceum on the energetics and feeding of Arctic charrSalvelinus alpinus. J Fish Biol 73:2228-2237. http:// doi.org/1.0.l l l l/j.l095-8649.2008.02050.x

Voutilainen A, Valdez H, Karvonen A, Kortet R, Kuukka H, Peuhkuri N, Piironen J, Taskinen J (2009) Infectivity of trematode eye flukes in farmed salmonid fish - Effects of parasites and host ongms. Aquaculture 293:108-112. http://dx.doi.org/10.10l6/j.aquaculture.2009.04.006

Woo PTK (2006) Fish Diseases and Disorders, Volume 1, Protozoan and Metazoan Infections. 2nd Edition, CAB International, pp. 348 - 352