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SEASONAL VARIATION IN THE INFESTATION OF DIGENEA IN THE INDIAN KILLIFISH *Aplocheilus lineatus*

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ABSTRACT

An overview of digenean trematode parasitology from the native killifish ***Aplocheilus lineatus*** is presented, cluding a discussion of the "design-like" features of these parasites .Infections of cercariae were found during the course of reproductive study of *Aplocheilus lineatus* a native killifish collected from the River Tamparabaranei. The frequency of occurrence and their effect on the killifish were calculated. It is observed that a large number of cercaria larvae were found in the liver than in the ovary of *Aplocheilus lineatus.* Testis were completely devoid of digenea. The fecundity was less in the infected fish than in a healthy normal individual.

Keywords: *Aplocheilus lineatus*, cercaria, digenea, trematode, Ascocotyle sps.

1.INTRODUCTION

A wide range of metazoan parasites are known to infect the fishes from the wild as well as artificial habitats. Digenetic, heterophyid trematode parasitic worm of the genus *Ascocotyle* infects certain snails as first intermediate hosts (*Littoradinops*) . They also infect certain cyprinodont and poeciliid estuarine fishes (*Cyprinodon, Poecilia, Fundulus, Gambusia, Aplocheilus*), as second intermediate hosts in a three-step life cycle, (Lumsden and Armitage, 1999). The apparent pathogenicity of the *Ascocotyle* genus have been reported by Stunkard and Uzman, 1955; Burton, 1956; Lenhoff *et al*., 1960; Lumsden, 1963a; Schroeder and Leigh, 1965; Martin and Steele, 1970; Skinner, 1975; Font, Overstreet and Heard, 1984; Snyder *et al*., 1989; Font, Heard and Overstreet, 1984.; Coleman, 1993; Ostrowski de Nunez, 1993, Kilian and Oldewage, 2013; Santos and Santos, 2013 and Renick *et al.*,2016 reported that though number of parasites were more no pathogenicity was observed.

The pathogenicity of metazoan parasites depends on the nature of the parasite, its density, host resistance and availability of vectors. The pathological symptoms include growth retardation, tissue destruction, metabolic disturbances, nervous disorders and even death in heavy infections. Control of metazoan infections relies on the better understanding of their biology, ecology and host parasite interactions.

The present study pertains to the clinical signs, frequency of infestations, pathogenesis and gross pathological changes in *Aplocheilus lineatus*.

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2.MATERIAL AND METHODS

Study area: The study was carried out in the River Tambraparnei (8° 30' N - 70° 45' E) situated at the heart of Tirunelveli District, India which has a vast freshwater biodiversity.

Study design: A total of 2400 live specimens of *Aplocheilus lineatus* were collected using a scoop net (2mm mesh size) net from the River Tambraparnei for a period of 12 months (August 2008 to July 2009) and were then transported to Animal Health Research Unit, St. Xavier's College, Palayamkottai. All these fishes were transferred to cement tanks and the investigation was carried out.

Parasitological techniques: At the laboratory fishes were sacrificed. Every fish was dissected and their gonads, liver, intestine and brain were examined under a dissection microscope. The fishes showing infestation of digenean larvae were recorded separately from the uninfected fishes. The fecundity, weight of the gonad, weight of the liver, no.of parasites present in liver and gonads and morphometric

measurements such as standard length, total length, length of the ovary, were recorded separately.

3.RESULTS

Every month more than 250 fishes were examined for parasites internally. Data was recorded sex-wise every month throughout the study period. Monthly record of the trematode parasite *Ascocotyle sps*. infestation in both the sexes is tabulated in Table- 1. It is observed that more number of males was infected than females. Ascocotyle sps infestation was observed in the liver and ovary of *Aplocheilus lineatus.* Table 2 shows the number fishes infected in the liver, testis and ovary.

Infestation of the digenean parasite *Ascocotyle* was observed only in the liver and ovary of *Aplocheilus lineatus* for females. Only the gonads of mature females were infected. In the case of males, infection was observed only in the liver and not in the testis. It was observed that there was seasonal variation in the frequency of infestation of the digenean parasite in *Aplocheilus lineatus*. Even though there is not much difference in the weight of the ovary of an infected and uninfected fish, fecundity of the infected *Aplocheilus lineatus* was less when compared with an uninfected fish (Table-3). It was also noted that infestation at the ovary starts only after the fish becomes sexually mature (45mm total length). Table 4 shows the statistical analysis of variance between the infected and the normal *Aplocheilus lineatus*. The p value was < 0.05. Hence the variation between male and female infestation of *Ascocotyle sps* in *Aplocheilus lineatus* is significant.

Table 1: Month wise prevalence of digenea in *Aplocheilus lineatus*

Period	No. of fishes examined	No.of fishes infected	% of infection	No. of infected female fishes	% of infection	No.of infected male fishes	% of infection
August 2002	250	79	31.6	23	41.7	46	58.3
September 2002	255	90	35.2	35	38.8	55	61.2
October 2002	220	87	39.5	43	49.4	44	50.6
November 2002	289	121	41.8	59	48.7	62	51.3
December 2002	210	95	45.2	38	40	57	60
January 2003	295	35	11.8	11	31.4	24	68.6
February 2003	345	46	13.3	18	39.1	28	60.9
March 2003	231	29	12.5	14	48.2	15	51.8
April 2003	250	45	18.0	10	22.2	35	77.8
May 2003	283	67	23.6	28	41.7	39	58.3
June 2003	322	82	25.4	39	47.5	43	52.5
July 2003	331	98	29.6	42	47.8	56	52.2

Table -2. Parasites recovered from liver and gonad of *Aplocheilus lineatus* sex-wise

Period	Infected fishes	Males		*Females	
		Liver	Testis	Liver	Ovary
August 2002	79	46	-	23	10
September 2002	90	55	-	35	7
October 2002	87	44	-	43	15
November 2002	121	62	-	59	11
December 2002	95	57	-	38	13
January 2003	35	24	-	11	2
February 2003	46	26	-	18	5
March 2003	29	15	-	14	7
April 2003	45	25	-	10	9
May 2003	67	39	-	28	13
June 2003	82	43	-	39	20
July 2003	98	56	-	42	15

Table 3. Lengthwise fecundity of infected and healthy *Aplocheilus lineatus.*

Standard Length (mm)	Gonad wt of infected	Absolute fecundity of infected	Gonad wt of uninfected	Absolute fecundity of uninfected
35 - 45	0.027	188	0.024	213
46 - 55	0.038	221	0.035	258
56 - 65	0.045	265	0.043	312
66 - 75	0.063	273	0.062	323

Table 4. ANOVA of infected and normal *Aplocheilus lineatus*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	266499.2	3	88833.07	85.0501	2.36E-08	3.4903
Within Groups	12533.75	12	1044.479			
Total	279033	15				

4.DISCUSSION

The infected fish underwent stress and showed undulatory movements which enable the predators like Herons, Storks and Crane to catch the fish due to the silvery shinning of the abdomen. Fishes held under adverse conditions in the aquarium showed endocrine imbalance, cellular hyperplasia and lymphoid evolution. Stress induced due to the infestation affects the immune response (Wedemeyer, 1970; Kilian and Oldewage, 2013; Santos and Santos, 2013; Renick *et al*., 2016). Lesions on the gills associated with parasitic infection vary with the agent, host and destiny of infection.

Ovarian changes during the reproductive cycle may be inhibited by disease or malnutrition and result in atresia or failure to develop of primary oocytes. Digenean trematodes have a complex life cycle involving a series of hosts. Fish is the primary or intermediate host depending on the digenean species. They are found externally or internally, in any organ. For the majority of digenean trematodes, pathogenicity to the host is limited. Trematode infection begins as a horn snail grazing on algae incidentally ingests worm eggs, perhaps from a bird dropping. The eggs hatch into worms that prevent the snail's own reproduction. Instead, the infected snail nourishes the growing larval worms, which eventually develop into a free-swimming stage and leave the snails to seek their second, or intermediate, host.

Depending on the worm species, the intermediate host might be a crab, fish, bird or another species of snail (Kevin Lafferty 2002). The most common salt marsh trematode infects California killifish. By traveling to the fish's brain, the worm causes the fish to behave differently from other killifish. An infected fish will sometimes move about jerkily near the water's surface, turning on its side and flashing its light-colored belly. This behavior attracts predators like herons; capturing infected fish is 10-30 times easier for the crane than capturing healthy fish (Kevin Lafferty 2002).

The crane in turn becomes the host to the adult worm, Kevin Lafferty (2002). The adult trematode takes up final residence in the bird's gut, releasing thousands of eggs that are deposited in bird droppings back into the salt marsh, completing the life cycle of the parasite.

Most trematodes must infect three different "host" animals – first, intermediate, and final to develop into egg-producing adults. Thus, if one of its host animals is missing, as it may be in a degraded ecosystem, the parasite cannot complete its life cycle (Kevin Lafferty, 2002; Santos and Santos, 2013; Renick *et al*.,2016).

In 1999, Lumsden and Armitage, Kilian and Oldewage, 2013 and Renick *et al*.,2016 reported about the digenetic trematode *Ascocotyle* infecting certain cyprinodont and poeciliid estuarine fishes (*Cyprinodon, Poecilia, Fundulus, Gambusia*), as second intermediate hosts. The *Ascocotyle* group comprises some 30 different species, which vary by mostly minute morphological differences, such as spine count and shape, organ position and size, and organ shape within adults. They also vary in metacercarial cyst shape and thickness, location of infection within the second intermediate host, and the specific host type.

In 1962 Olsen observed the presence of black grub, white grub and yellow grub in the fishes in earthen ponds located in North Central Region of United States. Black grubs are frequently see in freshwater fishes as pinhead-sized black spots in the skin, tail base, fins and musculature (Hunter and Hunter,1938; Santos and Santos, 2013).

White grubs primarily affect kidneys, liver and heart, but they also occur in spleen, connective tissue of the gut and ovary (Avault and Simtherman 1965; Spall and Summerfelt 1970).

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