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RESEARCH ARTICLE

SPATIAL DISTRIBUTION OF GILL MONOGENEAN PARASITE, *DACTYLOGYRUS FOTEDARI* GUSEV, 1973 FROM *LABEO CALBASU* HAMILTON, 1822 IN YSR KADAPA DISTRICT, ANDHRA PRADESH, INDIA

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ABSTRACT

A parasitic survey was conducted from March 2017 to March 2018 to highlight the spatial distribution of the gill monogenean, *Dactylogyrus fotedari* Gusev, 1973 from *Labeo calbasu* Hamilton, 1822 of different localities of YSR Kadapa District, Andhra Pradesh using routing parasitological techniques. A total of 2123 *Dactylogyrus fotedari* Gusev, 1973 was obtained from the gills of these 122 fishes with a prevalence of 75.4%, mean intensity of 23.07 and mean abundance of 17.4. The parasite showed an aggregated distribution pattern (70.04). There was a negative but weak relationship between the intensity of infection and the relative condition factor. The parasite exhibited seasonal fluctuation; the maximum intensity of parasite infection was recorded in winter and minimum in the summer season. The small sized fishes ranging between 80-100 mm were showed high parasitization followed by the large sized fishes ranging from 120-140 mm, whereas the medium sized fishes showed minimum infection. The host sex has no significant effect on the parasitization. No specific preference for the right or left side of the fish host was observed and the parasite species colonized the middle arches (Gill arch II and III). This type of studies will help the aquculturists to implement many advanced aquaculture practices and increase the productivity of *Labeo calbasu* in the Southern states of India.

Key words: *Dactylogyrus Fotedari*, *Labeo Calbasu*, Spatial Distribution, Prevalence, Mean Intensity, Aggregated Distribution.

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INTRODUCTION

Fish is the primary source of food especially rich in high quality of proteins also contains lipid, minerals, oils and vitamins. Due to an increasing demand for fish, practices of aquaculture have been intensified consequently. But, unfortunately, aquaculture has been facing various hazards with virus, bacteria, fungi and parasites (Puinyabati *et al*., 2010). Monogeneans are one such largest group of parasitic platyhelminthes parasitizing mostly fishes and other lower aquatic invertebrates as ectoparasites (Reed *et al*., 2012). These monogeneans preferably assault the various body parts such as gills and skin but also invade body cavity, rectal cavity, intestine and even vascular system (Rohde *et al*., 1992; Whittington *et al*., 2000). All monogeneans are oviparous except gyrodactylids. Monogeneans are a great havoc to the fishes and other hosts as they cause localized hyperplasia, osmoregulatory disturbances and mortality of the hosts (Piasecki *et al.,* 2004; Blahoua *et al*., 2015, 2016, 2017, 2018) and severely affect its commercial value of fish around the world (Bichi and Ibrahim, 2009).

Labeo calbasu Hamilton, 1822 commonly known as 'Black rohu' is one of the major Indian carps next to the three Indian major carps i.e *Labeo rohita,Catla catla and Cirrhinus mrigala* occurring very commonly in the various rivers of India and adjacent countries (Chondar 1999). This fish has a high market value due to its good taste, less intra muscular bones, high protein value and its liver oil being a good source of Vitamin-A (Ghosh *et al*., 1933). It is a common game fish in the tanks where it is usually stocked and cultivated along with other fish species (Talwar and Jhingran, 1991, Rahman, 2005). This fish also plays a pivotal role as scavengers feeding on dead and decaying matters at the bottom of the tanks and improving the sanitation of the tanks (Bhuiyan, 1964). *L.calbasu* is also sold as ornamental fish in the fish markets of India (Gupta *et al*., 2012) and also has been be exported from India as native ornamental fish (Gupta and Benarjee. 2014). Although much has been done on the taxonomy and biology of the monogeneans in India (Chaudhary *et al*., 2013, Sujana and Shameem, 2015, Gudivada *et al*., 2017), but the information on the infection dynamics and spatial distribution of monogeneans from fishes are sparse (Ramasamy and Ramalingam, 1989; Agrawal and Mishra, 1992; Tripathi *et al*., 2010; Kumar *et al*., 2017). Monogeneans are mostly restricted not only to a particular host but also to a particular part of their host's body (Turget *et al*., 2006).

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However, in the present study a genuine attempt was made to study the spatial distribution of the gill parasite, *D.fotedari* on the gills of the Black rohu, *Labeo calbasu*.

MATERIALS AND METHODS

Study area: The Black carps (*Labeo calbasu*) were collected from local fisherman from three different sites of YSR Kadapa District (Lat. 14°28´N 78°49´E, 137 m Altitude), located in Andhra Pradesh state during February, 2013 to February, 2015. The collection sites include (Fig-1):

Site 1: Mylavaram Reservoir across the Penna River in Mylavaram village (Lat.14° 0′ 15″N 78° 20 40″ E longitude), located in YSR Kadapa District of Andhra Pradesh.

Site 2: Aadinimmayapalle Dam across the Penna River in Chennur Village (Lat.14″34′0.12″N, 78″48′ 0″E longitude), YSR Kadapa district.

Site 3: Backwaters of Somasila reservoir across the Penna River in Somasila village (14°29′22″ N 79°18′19″E) Nellore District, Andhra Pradesh reach near Vontimitta Village, Kadapa.

***Fish sampling and Parasitological examination*: A** total of 122 *Labeo calbasu* specimens weighing 120-1000g were collected from the local fishermen from the three fish sampling locations for a period of 13 months (March, 2017 to March, 2018). Fish samples of different sizes i.e., small, medium and large were transported to laboratory. The morphometrics such as total length (mm), standard length (mm) and weight (g) were recorded for each fish specimen before dissecting the fish. The sampled fishes were categorized into three size classes of 20mm amplitude (Class-I: SL ranged between 80-100mm; Class-II: SL ranged between 100-120mm; Class-III: SL ranged between 120-140mm).

The effect of the parasites on the health condition was calculated from the Fulton's condition factor (K-factor) with the following formula: $K_c = W \times 10^5/SL^{3,}$ W is the weight (grams) and SL the standard length of fish (millimeters) (Klemm *et al*., 1992). Fishes were dissected to determine the sex and the external surfaces such as gills, fins, scales, eyeballs and buccal cavity were meticulously examined for the presence of ectoparasites. The operculum of the fish was removed to expose the gills, which were carefully removed fresh, separated into left and right, and stored in ice (0ºC). The left and right gill arches excised were separated, and placed in a petridishes containing water. Gill arches were observed using a stereozoom microscope (LM-52-3621 Elegant). Gill arches from both sides of the fish were numbered I to IV from the anterior gill arch below the operculum to the posterior. The surface of each hemibranch was represented as outer (surface nearer to operculum) and outer (Turgut *et al*., 2006) and each gill was arbitrarily divided into 4 sections: 1, 2, 3 and 4 obtaining 16 sectors from four gill arches of one side (Shaharom, 1985; Dzika, 1999). The number of monogeneans on each sector was collected and recorded with their positions is clearly depicted in gill map (Fig.2) Monogeneans were too small to prepare permanent slides, hence temporary slides were prepared using neutral red and ammonium picrate-glycerine mixture, following the method of Malmberg (1970). Neutral red ($C_{15}H_{17}N_4Cl$) helps to study the reproductive organs details and ammonium picrate-glycerine mixture was used to study the sclerotised hard parts of the parasite. Observed parasites were identified using Gusev (1976, Pandey and Agrawal, 2008). The temporarily stained parasites were observed and identified under the Lynx trinocular microscope (N-800M) and their microphotographs were captured and line diagrams were drawn with the aid of attached drawing tube.

Data analysis: The standard parasitological terms (Prevalence, mean intensity, mean abundance and index of infection were followed according to Bush *et al*., (1997). Distribution patterns of the parasite were determined using dispersion index (S^2/x) (Poulin, 1993; Shaw and Dobson, 1995). Karl Pearson's correlation coefficient (r) and Spearman's rank correlation coefficient (r_s) was used to test the possible relationship between the host size, host age and overall parasitization (Zar, 1996). Mann-whitney U-test was used as an indication to scrutinize the influence of host sex on the parasitic abundance (Vassarstat.net/utest.html). The test statistics were computed using Microsoft Excel 2007 and IBM SPSS 21.0 version with a statistical significance level of $p \leq 0.05$.

RESULTS

Temporal variation on the occurrence of the parasites: *Dactylogyrus fotedari* was obtained during the entire study period from the gills of *L.calbasu.* Prevalence, mean intensity and dispersion index of *D.fotedari* is given in Table-1. The data reveals that *D.fotedari* has adopted an aggregation distribution. The parasite showed a positive and statistically significant correlation with the condition factor of the fish (r_s = 0.83) (Table-2). *D. fotedari* occurred in the host population in almost all the months with a highest prevalence (100%) in October 2017, November, February, 2018 and March, 2018 and least in the month of April, 2017 (10%) followed by March, 2017 (25%) and January, 2018 (25%) (Table-3, Fig.2). There was a significant difference in the prevalence and mean intensities between the seasons (Analysis of variance, F= 3.69, p =0.029). Parasitism changed from one season to another. Only the parasitization in summer and winter showed a statistically significant difference (t = -5.30, p = <0.000). Parasitization was highest in winter (93.2%), followed by rainy (87.5%) and least in Summer (42.1%) (Table-4).

***Distribution and prevalence of D.fotedari from different localities* (Table-5):** The infestation rate of *D.fotedari* was high in fishes collected from back waters of Somasila reservoir with prevalence and mean intensity of 85.4% and 28.7 respectively and least in fishes collected from Mylavaram reservoir.

Spatial distribution of D.fotedari on gills: The prevalence and mean intensity values of *D.fotedari* were 67.2% and 9.43 on the left side and 74.6% and 14.8 on the right side of fish respectively (Table-6). These values were statistically significant at 5% (χ^2 = 8.19, p =0.0042, df =1; analysis of variance, F = 0.06, p = 0.0023). This species was more frequent and concentrated on gill arches I and II (χ^2 = 7.75, p =0.051, df =1; analysis of variance, F = 3.16, p = 0.0247) and least on arch IV.

Relationship between body length and degree of infection: The infestation rate of *D.fotedari* was highest in the Class-I with SL ranging between 80-100 mm and Class-III with SL ranging between 120-140 mm and low in the medium sized fishes.

Table 1. Prevalence, mean intensity, mean abundance, index of infection and dispersion index of *D.fotedari* in *L.calbasu*

Parasite species	Prevalence (%)	Mean intensity	Mean abundance	Index of infection	Variance	Dispersion Index (S^2/x)
Dactylogyrus fotedari	75.41	23.07	17.4	13.1	1218.9	70.04 (Aggregated)

Table 2. Values of Spearman's rank correlation coefficient, r_s correlating the relative condition factor and the abundance of *D. fotedari* parasitization in *L.calbasu*

Parasite species	r_s	*P*
D. fotedari	0.83*	0

*= Significant level at p< 0.05

Table 3. Monthly population dynamics of *D.fotedari* from the gills of *L.calbasu*

Months	No. of fishes examined	No. of infected fishes	No. of parasites	Prevalence (%)	Mean intensity	Mean abundance	Index of infection
March, 2017	8	2	27	25	13.5	3.375	0.844
April	10	1	4	10	4	0.4	0.04
May	10	5	21	50	4.2	2.1	1.05
June	10	8	172	80	21.5	17.2	13.76
July	10	9	70	90	7.78	7	6.3
Aug	10	8	58	80	7.25	5.8	4.64
Sep	10	8	508	80	63.5	50.8	40.64
Oct	10	10	170	100	17	17	17
Nov	10	10	320	100	32	32	32
Dec	10	10	199	100	19.9	19.9	19.9
Jan, 18	4	1	3	25	3	0.75	0.188
Feb	10	10	309	100	30.9	30.9	30.9
Mar	10	10	262	100	26.2	26.2	26.2

Table 4. Seasonal changes of prevalence (%), mean intensity and mean abundance of *D.fotedari* in *L.calbasu*

Parasite species	Seasons	No. of fishes examined	Range	Prevalence (%)	MI	F	p-value	Comparison two by two	t	p-value
D.fotedari	Summer	38		42.1	14			Summer-Rainy	-1.54	0.063
	Rainy	40		87.5	23			Summer -Winter	-5.30	<0.00*
	Winter	44		93.2	26.7	3.69	0.029	Rainy-Winter	-0.533	0.29

*= Significant level at p< 0.05

Table 5. Prevalence, mean intensity, mean abundance and index of infection of *D.fotedari* in *L.calbasu* collected from different locations

Collection sites	Total no. of fishes	Infected fishes	Total no. of parasites	Range	Prevalence (%)	Mean intensity	Mean abundance	Index of infection
Chennur	57	37	598	2-73	64.9	16.1	10.49	6.81
Mylavaram	10	08	172	6-69	80	21.5	17.2	13.76
Somasila	55	47	1353	1-346	85.4	28.7	24.6	21.02

Table 6. Prevalence and mean intensity of *D.fotedari* infection in relation to host side

Parasite species	No. of fishes examined	Prevalence (%)		MI		MA		II	
		Left side	Right side	Left side	Right side	Left side	Right side	Left side	Right side
D. fotedari	122	67.2	74.6	9.43	14.8	6.34	11.0	4.26	8.24

Table 7. Prevalence and mean intensity of *D.fotedari* as a function to the gill arch

D. fotedari	Gill arch I	Gill arch II	Gill arch III	Gill arch IV
No. of infected fishes	86	83	84	76
No. of parasites	686	567	468	402
Range	1-104	1-122	1-70	1-50
Prevalence	70.5	68.0	68.9	62.3
Mean Intensity	8.0	6.8	5.6	5.3
Mean abundance	5.6	4.6	3.8	3.3
Index of infection	4.0	3.2	2.6	2.1

Table 7. Prevalence and mean intensity of *D.fotedari* in relation to host size

Parasite species	Host length classes	No. of fishes examined	Prevalence (%)	Mean intensity	F	p-value	Comparison two by two	t-value	p-value
D. fotedari	Class-I	57	84.2	29.8	2.86	0.0612	Class-I-II	2.03	0.225*
	Class-II	36	61.1	14.2			Class-I-III	1.34	0.092
	Class-III	29	75.8	17.0			Class-II-III	-1.10	0.137

Table 8. Prevalence, mean intensity, mean abundance and index of infection of *D.fotedari* in *L.calbasu* in relation to host sex

Sex of species	a	b	c	Range	Prevalence (%)	Mean intensity	Mean abundance	Index of infection	Z-value
Females	31	20	522	3-72	64.5	26.1	16.8	10.86	0.499
Males	91	72	1601	1-346	79.1	22.23	17.5	13.9	p=0.617

a= No. of fishes examined; b= No. of infected fishes; c= No. of parasites

Site 1. Mylavaram Reservoir across the Penna River in Mylavaram village, YSR Kadapa District

Site 2. Aadinimmayapalle Dam across the Penna River in Chennur Village, YSR Kadapa district

Site 3: Backwaters of Somasila reservoir across the Penna River in Somasila village, Nellore District, Andhra Pradesh reach near Vontimitta Village, Kadapa.

Fig.1. Geographical location of the three fish sampling sites of YSR Kadapa District, Andhra Pradesh

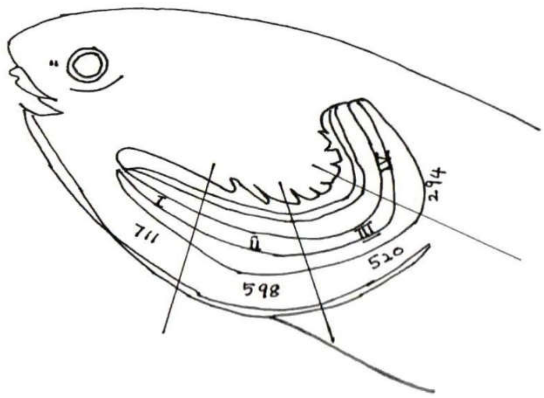

Fig. 2. Diagram exemplifying site-specificity of *D.fotedari* along the anterio-posterior axis of the host gills (I-anterior region; II=anterior middle region; III-posterior middle region; IV-posterior region). The figure represent the total number of parasites recorded from 122 *L.calbasu*

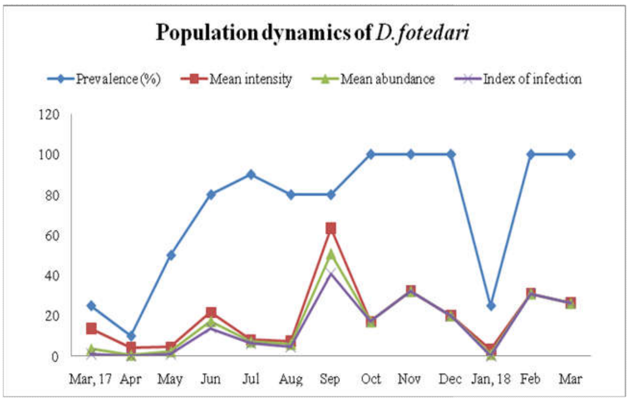

Fig. 3. Monthly population dynamics of *D.fotedari* infection in *Labeo calbasu*

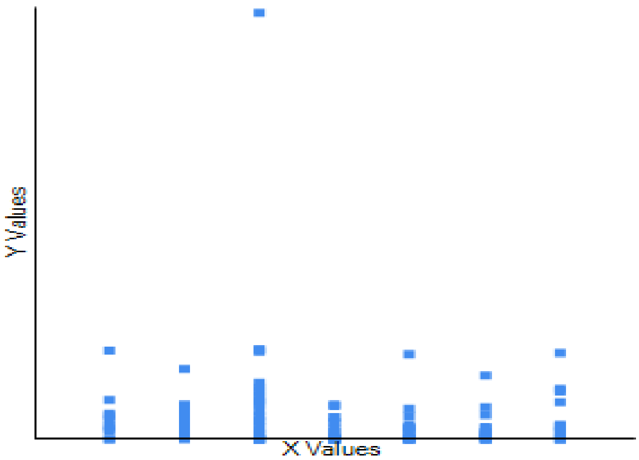

Fig.4. Correlation of host size with the overall parasitization of *D.fotedari*

Statistical tests showed significant differences in the prevalence according to size classes ($\chi^2 = 9.95$, p =0.0068, df =2; analysis of variance, F = 2.86, p = 0.061). Student's t-test also revealed that there is a significant difference from Size class-I and II (Student 't' = 2.03, p = 0.225) while the remaining size classes did not show any significant differences (Table-7).

Relationship between host sex and degree of infection: The prevalence of *D.fotedari* was 79.1% for male fish and 64.5 % for female fish. Female fishes showed slightly higher mean intensity than the males (26.1). Host sex has no significant affects on the parasitic infection (z = 0.499, p = 0.617) (Table-8).

DISCUSSION

Parasites represent more than half of the total biodiversity on earth (Toft, 1986). Monogeneans are the most abundant group of helminth parasites in the aquatic environment (Ivona, 2004). They are predominantly ectoparasitic on gills and skin of fishes with relatively high host specificity. These fish parasites would cause for different variety of damages like irritation, wound, injury or atrophy of tissue and occlusion of the alimentary canal and blood vessels (Bedasso, 2015). Buchmann and Bresciani (2006) assumed that many fish hosts including freshwater ones could harbor atleast one unique monogenean species.

Monogeneans are mostly restricted not only to a particular host but also to a particular part of their host's body such as gills and skin (Turgut *et al*., 2006). They also show a specific inclination in the different parts of the gills of the host fishes (El-Hafdi *et al*., 1998; Dzika, 1999; Shaharom, *et al*., 1985). In the present study, *D.fotedari* is a frequently observed monogenean parasite of *Labeo calbasu* which showed specific preferences for particular branchial arches or certain parts of the gill apparatus such as gill arch I and II and least preference for the inner most gill arch IV. This may be due to the strongest flow of water currents across these portions of the gill filaments which make it an opportune niche for the settlement of the parasite (Wootten, 1974, Turgut *et al*., 2006). The variation in water currents over the different parts of the gill surfaces also serves as one of the crucial factor in determining the distribution of these monogeneans (Turgut *et al*., 2006). Most of the parasites show aggregated distribution in the hosts which indicates the heterogeneity in the relationship between the host and parasite populations (Combes, 1995).

The aggregated distribution increases the chances for the parasites to meet their partner for reproduction (Kennedy, 1977). In the present study, *D. fotedari* also showed an aggregated distribution. There are studies which correlate the parasitic abundance per host fish with the condition factor of the fish (Yamada *et al*., 2008; Blahou *et al*., 2016, 2017). Yamada *et al*., (2008) reported a positive and significant correlation of condition factor of cichlids with the parasitic abundance of monogeneans. There are also few studies which showed negative and significant correlation or no correlation between monogenean parasitic abundance and condition factor of host fish (Lizama *et al*., 2007, Tozato, 2011). According to Cone (1995), the positive and statistically significant correlation of the parasite with the condition factor of the fish might be due to the large size body and better health condition of the host. *D. fotedari* also showed a positive and statistically significant correlation with the condition factor of the fish, *L. calbasu.* The occurrence of *D.fotedari* was noticed throughout the year in the host fish. Hence, the fish is considered to be susceptible at any period during the year. The present study is in total agreement with the earlier studies of Blahoua *et al*., 2015, 2016 and 2017) who opined the omnipresence of the parasites in the hosts throughout the year. Similarly, the seasons also play a vital role in the transmission and occurrence of the parasite. The occurrence of *D. fotedari* showed its highest prevalence and mean intensities during the winter season and lowest during the summer season which might be attributed to the variations in the water temperatures during these seasons. Few studies revealed the importance of water temperature in controlling the parasitic abundance in the host fishes (Koskivaara *et al*., 1991; Simkova *et al*., 2001; Blahou *et al*., 2016, 2017). There are several other abiotic factors such as turbidity, alkalinity, hardness, electrical conductivity, concentration of suspended particles apart from temperature which might persuade the seasonal occurrence of parasites in the host fishes (Bilong, 1995). There are many studies which correlate the parasitic abundance with the size of the host. Few studies supported the fact that parasitization increased with the increase in size of the fish (Mierzejwska *et al*., 2006; Vankara, 2018a, 2018b, Vankara and Chikkam, 2015, Vankara *et al*., 2016, Tombi *et al.,*2014, Blahoua *et al*., 2016).

There are also contrasting results of Boungou *et al*., (2008) who opined that there is no influence of host size on monogenean parasitization. In this study, the occurrence of *D. fotedari* infestation was high in the middle class and larger size fishes which might be due to the fact that the larger branchial surface area provides a great area of infestation and strongest water currents passing through gills of large sized fishes offers a suitable condition for parasite settlement (Aydogdu *et al*., 2003; Tekin-Ozan *et al*., 2008). In the present study, no significant preferences were found in the distribution of *D. fotedari* on the gill arches between right and left sides of the host. However, right gill arches showed slightly high prevalence than left side. The preference of parasite to specific site of the host may be associated with the body symmetry of the parasites (Rohde, 1993).

Since, dactylogyrids are bilaterally symmetrical; it is very likely these monogeneans can have equitable distribution on both sides of the gills which have similar morphology and exposure to ventilation currents. The gill monogeneans were most preferentially found attached to the middle arches II and III as reported by Tombi *et al*., (2014), Le Roux *et al*., (2011) and Blahoua *et al*., (2016, 2018). A number of basic factors such as water currents, need to locate the mate easily might be one of the reasons to choose a specific niche or microhabitat by the monogeneans (Le Roux *et al*., 2011 and Blahoua *et al*., 2016, 2018). The middle arches are described to be the most hydrodynamically protected sites for the monogeneans (Lo and Morand, 2001; Madanire-Moyo *et al*., 2010). Monogeneans parasites have free living stages (eggs or larvae) and they can settle in the middle arches which have a stronger water currents and high respiratory water (Lo and Morand, 2001). Even the parasitic load and host immunity can also influence the microhabitat selection (Koskivaara and Valtonen, 1992, Guitierrez and Martorelli, 1994).

Conclusion

This study describes the infestation of monogeneans and their site specificity within the gills of a host in a natural water system.
This detailed information on spatial distribution of monogeneans within the host gill can perk up the aquaculture practices and raise the productivity of the *L.calbasu.*

Conflict of interest: The authors declare that they have no conflict of interest related to the work.

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Author's contribution

The first author was involved in collecting the fish samples and parasites, literature collection, whereas the statistical analysis, drafting the manuscript was done by the second author who is the Research supervisor.

Significance Statement: This study discovers the fact that the gill monogenean *D.fotedari* prefers causes a lot of mechanical damage to the gills of the host. These parasites affect the fish health. Hence, it is always suggestible to study the spatial distribution of the parasite which provides an idea to the fish aquaculturists to implement suitable techinques to remove the monogenean infestation.

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