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PREVALENCE AND SPATIAL DISTRIBUTION OF ECTOPARASITES IN *Wallago attu* OF RIVER PENNA, YSR DISTRICT, ANDHRA PRADESH, INDIA

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ABSTRACT

An ectoparasitic survey on fish *Wallago attu* (n = 95) of river Penna, YSR district, Andhra Pradesh (India) was executed from August, 2017 to February, 2018. A total of 3202 parasites belonging three ectoparasitic groups *viz*., three monogeneans - *Thaparocleidus indicus, thaparocleidus wallagonius, Mizelleus indicus,* one copepod - *Ergasilus malnadensis,* and one isopod - *Alitropus typus* were detected from the gills of the examined hosts. Their spatial distributions on the host gills were reported. Only *E. malnadensis, T. wallagonius* and *T. indicus* showed aggregate distribution while the remaining two species, *M. indicus* and *A. typus* showed random distribution. Only, *A. typus, T. wallagonius* and *T. indicus* showed positive and statistically significant correlation with the condition factor. Location wise ectoparasite analysis revealed the higher infestation rates in site-II Somasila backwaters, Vontimitta compared to site-I Chennur. Spatial distribution studies revealed that there is no significant preference for the sides of the host fish. Both the sides of gill arch were equally infected. However, they showed preference within the gill arches. Monogeneans preferred gill arches II, III and IV, whereas copepods and isopods preferred gill arches I and II. There was meagre effect of host size and host sex on the ectoparasitic infection. These types of spatial distribution studies give an accurate location of the parasites to apply the eradication measures and eradicate them efficiently in fish aquaculture.

Keywords: Ectoparasites, mean intensity, prevalence, spatial distribution

INTRODUCTION

Wallago attu is a commercially important, fast-growing cat fish with a great demand due to its high nutritive value. It is also famous as sports fish and ornamental fish inhabiting the larger parts of South and South-east Asia. *W. attu* or 'Valaga' as it is known locally in Southern India, has been studied comprehensively in India for its helminth parasites. *W. attu* fish farming is gaining commercial importance due to its high price and demand; however, they are infected with a wide range of helminth parasites including both ecto- and endo-parasites (Pandey *et al*., 2003; Tripathi *et al*., 2010; Abro *et al*., 2019). Ectoparasitic infection includes monogeneans, copepods, and isopods. These ectoparasites are highly susceptible to changes in the aquatic environment like that of their host. A parasite's habitat consists of both abiotic (temperature, pH, dissolved oxygen, etc.) and biotic factors (host age, host length, host sex, immune response, etc.). Most ectoparasites are host-specific but some are site-specific within their host (Turgut *et al*., 2006, Adou *et al*., 2017). Taxonomic and pathological research on the ectoparasites of *W. attu* have previously been reported (Agrawal and Mishra, 1992; Seenappa and Venkateshappa, 2000; Pandey and Agrawal, 2008; Chaudhary *et al*., 2013; Priya and

Singh, 2020; Rafeeq *et al.*, 2021; Vankara *et al.*, 2022). There were scanty reports on the spatial distribution and community ecology of ectoparasites within the gills of various fishes (Ramasamy and Ramalingam, 1989; Tripathi *et al.*, 2010; Sujana, 2015; Kumar *et al.*, 2017, Modi and Vankara, 2019; 2021), but there is no report on the spatial distribution of ectoparasites on cat fish, *Wallago attu*. The aim of present study was to analyze the bio-ecological aspects of ectoparasites of *W. attu* and assess the spatial distribution of ectoparasites within the gills of *W. attu* collected from river Penna flowing through two distinct locations of YSR district, Kadapa, Andhra Pradesh, India.

MATERIALS AND METHODS

Study area

The cat fish species *W. attu* were acquired from two sampling stations of YSR Kadapa district (Latitude 14°28´ 38.0424″N; Longitude 78°48´ 17.7552″E), Andhra Pradesh from August, 2017 to February, 2018. The two sampling sites were: Site-I: Aadinimmayapalle Dam across the river Penna in Chennur village (Latitude 14″34′0.12″N; Longitude 78″48′0″E), YSR Kadapa district, and Site - II: Backwaters of Somasila reservoir across the river Penna in Somasila village (Latitude 14°29′22″ N; Longitude 79°18′19″ E) Nellore district, Andhra Pradesh reach near Vontimitta village, Kadapa.

Fish sampling and parasitological examination

The cat fish *W. attu* (n = 95) of length range between 7-15 cm (mean = 11.52 ± 1.95 cm) and weight 150-500 g (mean = 293.15 ± 100.9 g) were collected from the two sampling sites. Fish samples of assorted sizes (small, medium, and large) were transported to research laboratory to carry out parasitological examination. The morphometric analyses 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 sorted into three size classes of 30 mm amplitude [class-I: SL (standard length of fish in mm) between 70-100 mm; class-II: SL between 100-130 mm; class-III: SL between 130-150 mm]. Fulton's condition factor (K-factor): $K_c = W \times 10^5 SL^{-3}$, wherein W = weight (g) and SL = standard length, was applied to study the influence of parasitic septicity on health status (Klemm *et al.*, 1992). Fishes were sexed and all the external surfaces of fish (gills, fins, scales, eyeballs, and buccal cavity) were precisely examined for the presence of ectoparasites. The fish operculum was carefully alienated to uncover and separate the gills into left and right sides and was stored in ice (0°C) momentarily. The excised left and right gill arches were placed in a petri-dishes containing water before observing under a stereo-zoom 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. Standard protocols for the preparation of temporary mounts using ammonium picrate-glycerin mixture and Neutral red ($C_{15}H_{17}N_4Cl$) were followed due to small and delicate size of monogeneans (Malmberg, 1970). Lynx trinocular microscope (N-800M) was used to observe, identify, and capture the microphotographs of parasites and line diagrams were drawn with the aid of attached drawing tube. The identity of parasites was accomplished using relevant literature (Gusev, 1976; Pandey and Agrawal, 2008); while the copepod parasites were fixed in 10% formalin and identified using relevant literature (Yamaguti, 1963; Pillai, 1985).

Data analysis

The common parasitological terms such as prevalence, mean intensity, mean abundance and index of infection described by Bush *et al.* (1997) were considered in this study. Based on the prevalence of parasites, the species were categorized as core species (prevalence >50%), secondary species (prevalence, 11-50%) and satellite species (prevalence <10%) (Koskivaara and Valtonen, 1992; Valtonen *et al.*, 1997). Dispersion index (S^2/x) was used to study the distribution patterns of parasites (Poulin, 1993; Shaw and Dobson, 1995). The probable association between host size, host age and overall parasitization was tested by Karl Pearson's correlation coefficient (r) and Spearman's rank correlation coefficient (r_s) (Zar, 1996). The influence of host sex on parasitic abundance was scrutinized by Mann-whitney U-test (*Vassarstat.net/utest.html*). Microsoft Excel 2007 and IBM SPSS 21.0 version with a statistical significance level of $p \leq 0.05$ were used to execute these statistical tests.

RESULTS AND DISCUSSION

The sex ratio of host population was 0.417 (28 females, 67 males). Among the 95 fish examined, 94 were infested with the parasitic load of 3202 gill ectoparasites ranging from 1 to 235 specimens per parasitized host. Five ectoparasites belonging to three groups; monogenean (*Thaparocleidus indicus*, *Thaparocleidus wallagonius* and *Mizelleus indicus*), copepod (*Ergasilus malnadensis*) and isopoda (*Alitropus typus*) were obtained from the gills of the host with an overall prevalence of 98.9%, mean intensity of 34.1 and mean abundance of 33.7, respectively (Fig. 1). Only *E. malnadensis* (P = 98.6%), *T. indicus* (P = 55.8%) and *T. wallagonius* (P = 56.8%) were core species, while the remaining two species, *M. indicus* and *A. typus* showed prevalence <10% and were classified as satellite species. The aggregative mode of distribution of parasites within the hosts enables the parasites to meet their partner for reproduction more efficiently (Kennedy, 1977). *E. malnadensis* (32.6), *T. wallagonius* (11.3) and *T. indicus* (17.8) showed an aggregated distribution pattern while *M. indicus* and *A. typus* depicted random distribution (Table 1). Buchmann and Bresciani (2006) and Morand *et al.* (2015) reported that the spatial niches of ectoparasites are restricted and parasite niches are segregated between the species.

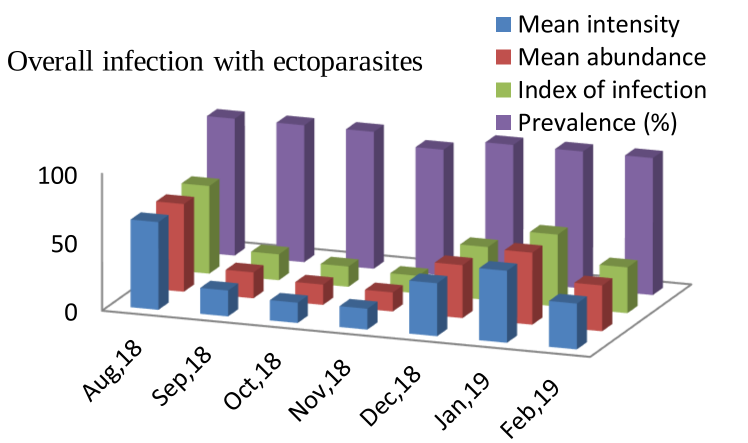

Fig. 1: Monthly population dynamics of ectoparasitic infection from the gills of *Wallago attu*

The condition factor of fish serves as one of the decisive factors in shaping the fish parasitic communities. The parasites, *A. typus*, *T. indicus* and *T. wallagonius* showed a positive significant correlation with condition factor of fish ($r_s = 0.83$) which correlates with the studies of Poulin and Morand (2000), Yamada *et al.* (2008) and Blahou *et al.* (2016, 2017); however, *E. malnadensis* and *M. indicus* showed only meager or no correlation with the condition factor (Table 2). The infestation rate of copepod, 3 monogeneans and isopod were high in fishes collected from site-II showed slightly high prevalence than left side for all the parasites, except *T. wallagonius* (Table 3). All the three groups

Table 1: Prevalence, mean intensity, mean abundance, index of infection and dispersion index of *ectoparasites* in *Wallago attu*

Parasite species	Prevalence (%)	Mean intensity	Mean abundance	Index of infection	Variance	Dispersion index (S^2/x)
Ergasilus malnadensis	96.8	22.8	22.1	21.4	719.7	32.6
Alitropus typus	9.5	1.1	0.1	0.0	0.111	0.1001
Thaparocleidus indicus	55.8	13.0	7.2	4.0	129.5	17.8
T.wallagonius	56.8	7.5	4.3	2.4	48.11	11.3
Mizelleus indicus	2.1	1.5	0.0	0.0	0.052	1.65

Table 2: Values of Spearman's rank correlation coefficient, r_s correlating the relative condition factor and the abundance of *ectoparasites* in *Wallago attu*

Parasite species	r_s	*P*
Ergasilus malnadensis	0.191	0.063
Alitropus typus	0.28208*	0.0054*
Thaparocleidus indicus	0.355*	0.00041*
T.wallagonius	0.436*	0.00001*
Mizelleus indicus	0	0

*= Significant level at p< 0.05

i.e. copepods, monogeneans and isopods were more concentrated in gill arches I (Somasila backwaters, Vontimitta) with prevalence of 98.4, 63.9 and 13.1%, respectively, as compared to the site-I (Chennur) [Table 4]. There was no significant preference for the distribution of ectoparasites on the gill arches between right and left sides of the host; however, right gill arches II and least on gill arches III and IV (Table 5, Fig. 2). To avoid interspecific niche competition, copepods and isopods inhabit outer arches I and II; while monogeneans inhabited the hydro-dynamically protective sites (gill arches II, III & IV). However, among the two monogeneans of same genus, *T. indicus* occupies gill arches II and III; while *T. wallagonius* occupies gill arches I and IV to avoid niche competition and correlated to the studies of Lo and Morand (2001), Madanire-Moyo *et al.* (2010), Le Roux *et al.* (2011) and Blahoua *et al.* (2018). The strongest inflow of water currents across the gill arches II, III and IV makes it an ultimate niche for the settlement of the parasites (Turgut *et al.*, 2006, Sujana, 2015; Zolovs *et al.*, 2018; Modi and Vankara, 2018, Gobbins *et al.*, 2021). Only, *E. malnadensis* showed statistically significant values at 5% ($\chi^2_{E.m}$ = 6.35, p = 0.0117, df = 1; analysis of variance, F = 4.64, p = 0.032); while the rest three monogeneans did not show significant values ($\chi^2_{T.i}$ = 1.25, p = 0.262, df = 1; analysis of variance, $F_{T.i}$ = 4.75, p = 0.0304; $\chi^2_{T.w}$ = 0.115, p = 0.734, df = 1; analysis of variance, F = 0.0028, p = 0.957; $\chi^2_{M.i}$ = 0, p = 1, df = 1; analysis of variance, F = 0.335, p = 0.563). The infestation rate of all these parasites was highest in the class-III with SL ranging between 130-160 mm followed by class-II with SL ranging between 100-130 mm and low in small size fishes. Statistical tests showed significant differences in the prevalence as per the size classes for *A. typus*, *T. indicus* and *T. wallagonius* ($F_{A.t}$ = 3291.43, p = <0.00001; $F_{T.i}$ = 4.36, p = 0.015; $F_{T.w}$ = 6.69, p = 0.0019); whereas

Table 3: Prevalence and mean intensity of ectoparasitic infestation from the gills of *Wallago attu* in relation to host side

Parasite species	No. of fishes infected	Prevalence (%)		Mean intensity		Mean abundance		Index of infection	
		Left side	Right side	Left side	Right side	Left side	Right side	Left side	Right side
Ergasilus malnadensis	92	94.7	95.8	9.34	13.8	8.85	13.21	8.38	12.7
Alitropus typus	9	-	9.5	-	1.1	-	0.1	-	0
Thaparocleidus indicus	53	37.9	52.6	6.86	8.9	2.6	4.7	0.98	2.46
T. wallagonius	54	45.3	41.1	4.67	5.07	2.12	2.08	0.95	0.86
Mizelleus indicus	2	1.05	2.11	1	1	0.021	0.0001	0.0001	0.0004

Table 4: Prevalence, mean intensity, mean abundance and index of infection of ectoparasitic infestation from the gills of *Wallago attu* 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
Copepods								
Chennur	34	32	432	1-30	94.1	13.5	12.7	12.0
Somasila	61	60	1664	2-234	98.4	27.7	27.3	26.8
Monogeneans								
Chennur	34	22	238	1-35	64.7	10.8	7.0	4.5
Somasila	61	39	858	1-75	63.9	22	14.1	9.0
Isopods								
Chennur	34	1	1	0-1	2.9	1.0	0	0
Somasila	61	8	9	0-2	13.1	1.1	0.1	0

Table 5: Prevalence and mean intensity of ectoparasitic infestation from the gills of *Wallago attu* as a function to the gill arch

Parameters	*E. malnadensis*				*A. typus*				*T. indicus*				*T. wallagonius*				*M. indicus*			
	Gill arch I	Gill arch II	Gill arch III	Gill arch IV	Gill arch I	Gill arch II	Gill arch III	Gill arch IV	Gill arch I	Gill arch II	Gill arch III	Gill arch IV	Gill arch I	Gill arch II	Gill arch III	Gill arch IV	Gill arch I	Gill arch II	Gill arch III	Gill arch IV
Infected fishes (No.)	90	90	88	85	6	4	0	0	40	40	39	40	39	30	24	37	1	1	0	1
Range	1-30	1-44	1-33	1-36	0-1	0-1	0	0	1-10	1-27	1-24	1-12	1-14	1-15	1-16	1-12	0-1	0-1	0	0-1
No. of parasites	601	608	491	396	6	4	0	0	157	202	194	136	123	86	77	118	1	1	0	1
Prevalence	94.7	94.7	92.6	89.4	6.31	4.21	0	0	42.1	42.1	41.1	42.1	41.1	31.6	25.3	38.9	1.05	1.05	0	1.05
Mean Intensity	6.67	6.75	5.57	4.65	1	1	0	0	3.93	5.05	4.97	3.4	3.15	2.87	3.21	3.19	1	1	0	1
Mean abundance	6.32	6.4	5.16	4.16	0.06	0.04	0	0	1.7	2.1	2	1.4	1.3	0.9	0.8	1.2	0	0	0	0
Index of infection	5.99	6.06	4.78	3.78	0.003	0.001	0	0	0.7	0.9	0.84	0.6	0.53	0.29	0.2	0.48	0	0	0	0

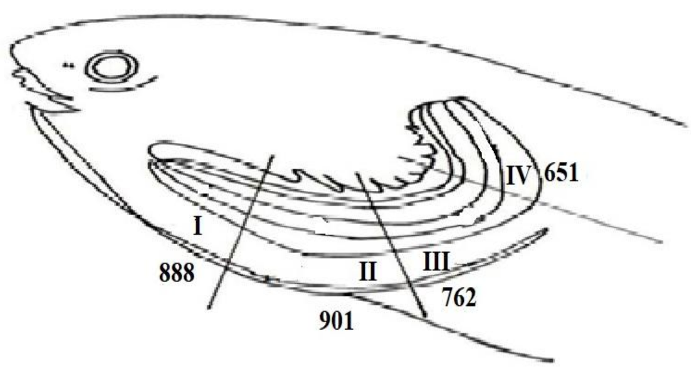

Fig. 2: Site-specificity of total ectoparasitic infection along the anterio-posterior axis of the host gills (I-anterior region; II=anterior middle region; III-posterior middle region; IV-posterior region) recorded from 95 *W. attu*

E. malnadensis and *M. indicus* showed a meager difference in their prevalence with size classes ($F_{E.m}$ = 1.91, p = 0. 154; $F_{M.i}$ = 0.356, p = 0.700). Student's t-test also revealed significant differences between size classes I-III and II-III for *A. typus*, *T. indicus* and *T. wallagonius;* whereas *E. malnadensis* and *M. indicus* did not show any significant differences between the size classes (Table 6). All the parasites depicted correlation between parasitic abundance and the size of host which is in line

Table 6: Prevalence and mean intensity of ectoparasitic infestation from the gills of *Wallago attu* in relation to host size

Parasite species	Host length classes	No. of fishes infected	Prevalence (%)	Mean intensity	F	p-value	Comparison two by two	t-value	p-value
E. malnadensis	Class-I (n = 17)	15	88.2	15.5	1.91	0.154	Class-I-II	-1.38	0.086
	Class-II (n = 41)	41	100	20.1			Class-I-III	1.54	0.063
	Class-III (n = 37)	36	97.3	28.9			Class-II-III	-1.22	0.112
A. typus	Class-I (n = 17)	0	0	0	3291.43*	<0.00001*	Class-I-II	-0.917	0.181
	Class-II (n = 41)	2	4.9	1			Class-I-III	1.850*	0.034*
	Class-III (n = 37)	7	18.9	1.14			Class-II-III	-2.018*	0.023*
T. indicus	Class-I (n = 17)	5	29.4	5.8	4.36*	0.015*	Class-I-II	-2.17*	0.0168*
	Class-II (n = 41)	22	53.7	11.6			Class-I-III	2.49*	0.0089*
	Class-III (n = 37)	26	70.3	15.5			Class-II-III	-1.70*	0.046*
T. wallagonius	Class-I (n = 17)	6	35.3	2.8	6.69*	0.0019*	Class-I-II	-1.738*	0.043*
	Class-II (n = 41)	21	51.2	5.8			Class-I-III	2.73*	0.004*
	Class-III (n = 37)	27	73.0	9.9			Class-II-III	**-2.62***	0.005*
M. indicus	Class-I (n = 17)	0	0	0	0.356	0.700	Class-I-II	-0.64	0.26
	Class-II (n = 41)	1	2.4	1			Class-I-III	-0.67	0.25
	Class-III (n = 37)	1	5.4	1			Class-II-III	0.51	0.25

*= Significant level at p< 0.05

Table 7: Prevalence, mean intensity, mean abundance and index of infection of ectoparasitic infestation from the gills of *W. attu* collected in relation to host sex

Sex of species	A	B	C	Range	Prevalence (%)	Mean intensity	Mean abundance	Index of infection	Z-value, P (2-tailed)
E. malnadensis									
Females	28	25	568	7-54	89.3	22.7	20.3	18.1	-0.269; p = 0.787
Males	67	67	1528	1-234	100.0	22.8	22.8	22.8	
A. typus									
Females	28	1	1	0-1	3.57	1	0.036	0.001	0.640; p = 0.522
Males	67	8	9	1-2	11.9	1.125	0.134	0.016	
T. indicus									
Females	28	14	179	3-31	50.0	12.8	6.4	3.2	0.310; p = 0.756
Males	67	39	509	1-62	58.2	13.1	7.6	4.4	
T. wallagonius									
Females	28	18	89	1-16	64.3	4.9	3.2	2.0	-0.216; p = 0.825
Males	67	36	316	1-41	53.7	8.8	4.7	2.5	
M. indicus									
Females	28	0	0	0	0.0	0.0	0.0	0.0	0.224; p = 0.825
Males	67	2	3	1-2	3.0	1.5	0.0	0.0	

A = No. of fishes examined; B = No. of infected fishes; C = No. of parasites; * = Significant level at p< 0.05

with Tombi *et al.* (2014), Vankara and Chikkam (2015), Blahoua *et al.* (2016) and Vankara (2018) as larger branchial surface area of large-sized fish and strongest water currents passing through them offer a favourable site for the settlement of young oncomiracidia and young naupli (Simková *et al.,* 2006). The inclination of parasites to specific site of the host may be related to the bilateral body symmetry of parasites (Rohde, 1993) and the bilaterally symmetry of parasites enable them to have equitable distribution on both sides of gills which have similar morphology and exposure to ventilation currents. Host sex has a vital role in the stability of host-parasite system (Lizama, 2007; Akhter, 2014).

The prevalence of *E. malnadensis* (100%), *A. typus* (11.9%), *T. indicus* (58.2%) and *M. indicus* (3.0%) were higher in male fishes as compared to the female fishes; however, the infestation of *T. wallagonius* was high in female fish than male fish. The values of z revealed that there was no significant effect of host sex on parasitic infection (Table 7). The infestation of parasites was higher in males than females. The variation in the infectivity levels of parasites in male and female hosts might be due to the variations in their physiological conditions to produce different quality and quantity of steroid hormones i.e androgens of males and estrogens of females. The values of Jaccard's index indicate that there is a strong interspecific association between *T. indicus* - *T. wallagonius* (3.28), followed by *T. indicus* - *E. malnadensis* (1.26) and *T. wallagonius* - *E. malndensis* (1.23); whereas the remaining parasites did not show much association (Table 8).

Table 8: Values of Jaccard's Index (JI) to estimate interspecific association between each pair of ectoparasitic infestation from the gills of *W. attu*

Parasitic species	*E. malnadensis*	*A. typus*	*T. indicus*	*T. wallagonius*	*M. indicus*
E. malnadensis	-	0.108	1.26	1.23	0.021
A. typus	0.108	-	0.14	0.142	NA
T. indicus	1.26	0.14	-	3.28	0.039
T. wallagonius	1.23	0.142	3.28	-	0.038
M. indicus	0.021	NA	0.039	0.038	-

NA = Not available

***Conclusion*:** These types of studies exemplify the invasion of ectoparasites and their site specificity within the gills of a host in native water systems. This comprehensive investigation on spatial distribution of ectoparasites within the host gills allows visualizing new protocols to monitor parasitic infections in intensive fish farming of the freshwater sharks, *W. attu* and raise their productivity.

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

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Significance Statement: These studies establish the fact that the ectoparasites cause a hysterical damage to the gills of the host, thus distressing the fish health indirectly. The study of the spatial allocation of the parasites and their impact on the fish will give a chance to fish aquacuturists to think alternatives to eradicate the ectoparasitic infestation.

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