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Lernaea Susceptibility, Infestation and its Treatment in Indigenous Major and Exotic Chinese Carps Under Polyculture System

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Abstract.- Present study was aimed to find out the *Lernaea* susceptibility, infestation and its treatment in indigenous major and exotic Chinese carps under polyculture system. 450 fishes were reared in the 0.5 acre ponds at Fish Pond Complex, Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus Pattoki and parasitic load was checked for the month of June, July and August. Five specimens of each species were caught on fortnightly basis and examined for the presence of *Lernaea cyprinacea* species on different body parts. The parasites were removed, preserved in 5% formalin and identified. Intensity of *Lernaea* was highest in the *Catla catla* followed by *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* in the month of June. Highest number of *Lernaea* (100) were counted in *Catla catla* and lowest in the *Labeo rohita* and *Cirrhinus mrigala* after the *Cyprinus carpio* which did not showed any parasite and behave as most resistant species among the Chinese and Indian major carps. Treatment with Thunder however, reduce the parasitic infection to a large extend and at the end of experiment all fishes were became free of parasite. Abdomen was the most prevalent site for the parasite attachment followed by the gills and caudal fins whereas head was the least.

Key words: *Lernaea*, Chinese carps, polyculture system.

INTRODUCTION

The ectoparasites of fish constitute one of the most important problems associated with pond fish culture. Some of them have really got lot of importance from parasitism point of view and therefore have become species of interest (Yin *et al.*, 1963; Kabata 1985; Tasawar *et al*., 2007a). Losses resulting from these parasites at high densities under existing conditions and further if left uncontrolled can reach catastrophic proportions. Lernaeidae is a major family of cyclopoid copepods associated with freshwater fish. Many species of the *Lernaea* (commonly called anchor worms) parasitize on freshwater fishes and have worldwide distribution. The adult parasites are particularly harmful to young fish because of their relatively large size and mode of attachment and feeding and lead to secondary bacterial infections when it leaves the host. Economic losses due to ectoparasite infestations not only result from direct harm to fish but also from disfigurement that inflict a big loss to fishing industry (Piasecki *et al.*, 2004). In Japan, the parasite has increased its number and spread to such an extent that it is a serious menace to fish culture. It was first found to be causing damage to eels choking the mouth cavity, but now it is also found on other fish and can be seen burrowing its head under the scales. Countless masses of fish have been destroyed because of these parasitic infections (Bauer *et al.*, 1973).

Lernaea is typically a freshwater copepod, but also parasitized brackish water fish on skin, gills, eyes, fins and even inside the mouth of fishes, near the palate and nostrils (Eiras, 1994). The female lernaeid copepod has long filamentous body with trailing attached egg sacs. Morphological modifications include the head, which is a rounded knob inserted into the musculature of its fish host with one or two pairs of anchors to hold it in position. Damage to the fish host includes hemorrhagic and ulcerated lesions with potential for secondary infections as anemia, retarded growth, loss of weight and balance of body. *Lernaea cyprinacea* has been recorded all around the world for example Europe, Scandinavia, France, Italy, Germany, Japan, Central Asia and West Siberia. The spread of *Lernaea cyprinacea* is limited by

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temperature as it is an exceptionally thermophilic organism of southern origin, and it develops successfully only at high temperatures (23-30°C).

Infestation of *Lernaea* species, are extremely difficult to control because only the free-living larvae are susceptible to treatment. The adult female produces three sets of eggs which hatch into larvae after four weeks. Since the larvae remain free living for about one week, it is necessary to treat once a week for four weeks to eliminate this parasite. Although the parasites infest throughout the year but high infestation intensity occurs only in the summer (Bauer *et al.*, 1973)**.** *Lernaea cyprinacea* is cosmopolitan species and is known to have worldwide distribution because of its peculiar quality of non-specific attacks which are generally absent in other members of its genus (Tidd, 1934; Uzmann and Rayner 1958) which has made it the most harmful parasite of cultured freshwater fishes (Hemaprasanth *et al*., 2008; Kabata, 1985; Molnar, 1987). Previous studies have revealed that *Lernaea cyprinacea* infection is a major disease threat encountered in carp culture in the Indian subcontinent and has been reported from *Catla catla*, *Labeo rohita*, *Cirrhinus mrigal*, *Hypophthlmichthys molitrix*, *Ctenopharyngodon idellus* and *Labeo fimbriatus* with variable intensity (Nandeesha *et al*., 1984, 1985; Tamuli and Shanbhouge, 1996; Iqbal *et al*., 2001a) and totally absent on common carp and *Labeo* calbasu from this region. Later on, experiments by Hemaprasanth *et al*. (2011) indicated infection in the common carp in polyculture system. Number of studies was carried out on *Lernaea* infestation, prevalence distribution and its effect on fish histology and hematology in different fish species individually, or in polyculture of carps. The present study was aimed to find out the prevalence and percentage distribution of *Lernaea* on different body parts in polyculture of Indian major carps and exotic Chinese carps in semi intensive culture at Fish pond Complex, Ravi Campus, Pattoki.

MATERIALS AND METHODS

Fish

Labeo rohita, *Cirrhinus mrigala*, *Catla catla* (local species), *Ctenopharyngodon idella;* *Hypophthalmichthys molitrix* (Chinese species), and *Cyprinus carpio* (exotic species) were used as experimental animals. Two groups of fishes namely control and treated were maintained. The treated group received regular applications of DDVP (@ 0.25 ppm), while the control group received no medication.

Two experimental ponds were treated with Tender, an organophasphate (DDVP or Dichlorvos - 2, 2-dichlorovinyl dimethyl phosphate) for the control of *Lernaea*. An insecticide dose was calculated using the following formula;

$$\text{DDVP (L)} = (L \times W \times D) \times 28.4 \times 0.25 / 10^{-6} = A \text{ (L)}$$
$$\text{Conversion into ml} = A \times 1000 = B \text{ (mL)}$$

B (ml) of tender were diluted tenfold with water and sprayed on surface of the pond water. Treatment was repeated after 3 days for three times.

Studies were conducted in 4 earthen ponds (59m× 30.5m× 1.8m) with two ponds per experimental group. Different fish species were randomly stocked in two replicates of each group following standard stocking procedures (Table I) and were fed isonitrogenous diet containing 40% crude protein formulated from fish meal, guar meal, soybean meal, cotton seed meal, corn gluten and canola meal @ 4% of their body weight twice a day.

Parasite collection, preservation and identification

Five specimens of each species were caught on fortnightly basis through net from experimental ponds, identified (Mirza and Sharif, 1996), kept alive in a plastic bowl and examined for the presence of *Lernaea* species. The parasites were removed with the help of fine forceps and preserved in vials containing 5% formaline. The parasites were permanently mounted and identified with the help of authenticated keys (Kabata, 1985).

The fishes were released back into their relevant ponds, after recording their weight and length.

Water quality parameters

Dissolved oxygen was monitored by DO meter, (YSI 55 Incorporated, Yellow Springs, Ohio, 4387, USA), pH (pH meter, LT-Lutron pH-207 Taiwan), electrical conductivity, water temperature,

salinity and total dissolved solids (TDS) by salinity meter (Condi 330i WTW 82362 Weilheim Germany) on daily basis. Chlorides (Mohr Argentometric method), phosphates ions (phosphate ion kit), nitrates (spectrophotopmetric determination) and secchi disk visibility were recorded fortnightly.

Statistical analysis

Results of infestation are expressed in percentages and one way analysis of variance (ANOVA) followed by Duncan Multiple Range test was used to evaluate the statistical differences of weight and length among the species by using SAS software version 9.1 version. Differences between the species means were considered significant at $P<0.05$).

RESULTS

Parasite identification

Microscopic examination of parasites, collected from fish of experimental ponds was identified as *Lernaea cyprinacea* according to Kabata (1985).

Weight and length gain in control and treated group

Lernaea cyprinacea susceptibility and infestation with treatment and without treatment with DDVP was studied during 90 days trial. Statistical analysis showed that maximum weight and length gain were observed in *Cyprinus carpio* followed by *Ctenopharyngodon idella, Hypophthalmichthys molitrix, Cirrhinus mrigala, Labeo rohita* and *Catla catla,* respectively in control group. In treated group similar trend was observed for weight gain however, trend in length gain was as followed *Cyprinus carpio, Labeo rohita, Hypophthalmichthys molitrx, Ctenopharyngodon idella, Cirrhinus mrigala* and *Catla catla,* respectively. Statistical analysis showed that there were significant differences between species weight and length at $P<0.05$ (Table I).

Infestation of Lernaea cyprinacea during the course of study in control and treated groups

Results showed the highest intensity of *Lernaea cyprinacea* in *Catla catla* followed by *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* however, it appeared that *Catla catla* developed resistance with the passage of time against parasitic infestation but persisted in other two species although, the number decreased considerably. Lowest numbers of *Lernaea cyprinacea* were counted in *Labeo rohita* and *Cirrhinus mrigala* that further decrease in *Labeo rohita* but increase in *Cirrhinus mirgala* in the month of August. Not a single *Lernaea cyprinacea* specimen was found on any part of *Cyprinus carpio* and emerged as the most resistant species among the Chinese and Indian major carps (Table II).

Similar trends were observed in treated group for all species. Maximum number of *Lernaea cyprinacea* was recorded on different parts of the body of fish in the month of June. Administration of DDVP reduced the parasitic infestation to a greater extent in the month of July and in August not a single fish showed lernaied infestation that indicates usefulness of thunder against the parasite (Table II). Number of parasites per fish and percentages of *Lernaea cyprinacea* on different body parts in control and treated groups were given in Tables III. Water quality parameters were remaining within the standard ranges required for the pond culture (Table IV).

DISCUSSION

Lernaea cyprinacea prevalence, susceptibility and its distribution was observed in Indian and Chinese major carps in polyculture, semi intensive system. Results indicated that *Catla catla* was the most susceptible species for *Lernaea cyprinacea* whereas it was totally absent in *Cyprinus carpio* and *Labeo rohita,* respectively. Our results were quite close to the report of Hemaprasanth *et al*. (2011) who concluded that *Cyprinus carpio* and *Labeo calbasu* showed low or no lernaeid infection under monoculture and poly culture, whereas, *Ctenopharyngodon idella, Hypophthalmichthys molitrix , Catla catla* and *Labeo fimbriatus* were susceptible under both type of culture systems showing their low resistance to the parasitic infection. *Labeo rohita* however, showed resistance in monoculture but could not maintain its resistance in polyculture practices. Even challenge with higher

Table I.- Fish stocked, weight and length gain in the control and treated group during the study period.

Species	n	Control group		Treatment Group	
		Weight gain (g)	Length gain (cm)	Weight gain (g)	Length gain (cm)
Catla catla	140	53.8±23.56^{c}	2.47±0.75^{c}	130.32±110.00^{c}	2.79±2.4^{e}
Cirrhinus mrigala	80	104.67±54.35^{c}	5.03±1.40^{bc}	212.22±78.95^{bc}	6.81±3.00^{d}
Ctenopharyngodon idella	80	385.67±123.56^{ab}	6.49±2.45^{b}	391.64±200.23^{ab}	6.03±0.98^{de}
Cyprinus carpio	60	500.34±324.95^{a}	13.3±1.50^{a}	550.00±117.67^{a}	19.1±0.17^{a}
Hypophthalmichthys molitrix	40	128.04±156.22^{bc}	5.02±1.30^{bc}	227.91±57.73^{bc}	10.72±2.10^{c}
Labeo rohita	50	72.73±98.56^{c}	3.71±2.10^{bc}	171.52±134.00^{bc}	14.5±1.80^{b}

Note: Figures with different superscript letters are significantly different from each other at $p<0.05$

Table II.- Average number of *L. cyprinacea* on different body parts during study period in the control and treated groups. (Mean±SD).

Study months	Species	Total no. of *L. cyprinacea*	Parasites on				
			Dorsal fin	Caudal fin	Abdomen	Head	Gills
Control groups							
June, 2010	*C. catla*	234	4.00±1.78	8.33±7.96	23.43±29.72	4.17±3.19	3.33±1.53
	C.mrigala	3	-	-	2.00±0.00	1.00±0.00	-
	C. idella	196	2.89±1.90	4.70±3.00	7.93±4.70	1.25±0.50	7.00±0.00
	C.carpio	0	-	-	-	-	-
	H. molitrix	109	1.0±0.00	4.67±6.35	10.38±22.14	1.33±0.58	7.00±0.00
	L. rohita	67	-	1.50±0.00	7.75± 0.00	1.00±0.00	-
July, 2010	*C. catla*	37	3.50±2.12	5.33±2.79	3.7±3.10	-	
	C.mrigala	10	2±0.00	-	4±2.82	-	
	C. idella	213	3.13±1.60	3.72±2.51	4.72±3.10	1.75±0.88	
	C. carpio	0	-	-		-	
	H. molitrix	16	3.5±0.71	1.00±0.00	2.33±0.57	-	
	L. rohita	3	-	-	2.00±0.00	-	
August, 2010	*C. catla*	6	-	-	-	2.00±1.41	-
	C. mrigala	52	4.00±1.00	4.50±0.71	6.67±5.13	3.67±0.58	-
	C. idella	23	-	5.00±0.00	6.5±0.71	-	-
	C. carpio	0	-	-	-	-	-
	H. molitrix	0	-	-	-	-	-
	L. rohita	14	-	1.67±1.15	3.00±1.73	-	-
Treated groups							
June, 2010	*C.*	119	1.33±0.57	4.5±5.39	8.44±10.13	3.33±1.52	2±0.00
	C. mrigala	36	-	1.2±0.00	2.0±0.00	-	-
	C. idella	59	1.10±0.44	1.6±1.34	1.05±1.59	-	-
	C. carpio	0	-	-	-	-	-
	H. molitrix	153	1.00±0.00	1.67±0.57	20.42±35.6	1.00±0.00	
	L. rohita	2	-	-	-	-	-
July, 2010	*C. catla*	15	-	1.00±0.00	1.20±0.42	1.00±0.00	-
	C. mrigala	0	-	-	-	-	-
	C. idella	37	1.25±0.50	3.67±0.00	1.92±0.90	1.00±0.00	-
	C. carpio	0	-	-	-	-	-
	H.molitrix	23	2.00±0.00	-	10.50±10.60	-	-
	L. rohita	0	-	-	-	-	-

Table III.- Number of *L. cyprinacea* and its %age on different body parts of experimental fish in the control and treated ponds during the course of study.

Species	n	Total no. of *L. cyprinacea*	No of parasite/fish	Number of parasites on (%)				
				Dorsal fin	Gills	Caudal fin	Abdomen	Head
Control ponds								
C. catla	59	500	9	31 (6.2)	10 (2)	153 (30.6)	275 (55)	31 (6.2)
C. mrigala	9	65	7	14 (22	0 0	9 (14)	30 (46)	12 (18)
C. idella	61	432	7	73 (17)	7 (2)	124 (29)	209 (48)	19 (4)
C. carpio	55	0	0	- -	- -	- -	- -	- -
H. molitrix	64	125	2	8 (6)	7 (6)	16 (13)	90 (72)	4 (3)
L. rohita	62	84	1	- -	1 (1)	8 (10)	73 (87)	2 (2)
Treated ponds								
C. catla	50	134	3	4 (3)	2 (1)	29 (22)	88 (66)	11 (8)
C. mrigala	14	36	3	- -	- -	13 (36)	23 (64)	- -
C. idella	66	96	1	11 (11)	- -	19 (20)	65 (68)	1 (1)
C. carpio	59	0	0	- -	- -	- -	- -	
H. molitrix	75	176	2	- -	5 (3	164 (93)	1 (1)	
L. rohita	69	2	0	- -	1 (50)	1 (50)	- -	

Table IV.- Mean values for water quality parameters during the course of study.

Parameters	Mean ± SD
DO (ppm)	5.9±0.8
pH	8.3±0.4
TDS (ppm)	1421.0±337.5
Salinity (ppt)	0.8±0.1
Temp.(°C)	31.6±1.9
EC (µS/cm)	2.1±0.1
PO_4^{-2} (ppm)	0.5±0.5
NO_3 (µg/L)	0.7±0.2
Cl^{-1} (ppm)	311.4±43.8
Secchi Disk Visibility(cm)	25.6±9.4

infective doses of copepodids under monoculture did not result infection in the resistant fish species. Ho and Kim (1997) also reported similar results *i.e.* prevalence of *Lernaea cyprinacea* was highest in *Catla catla* (26.67%) among 10 fresh water fish species, followed by *Lernaea polymorpha* (25.83%), *Lernaea oryzophila* (4.17%) and *Lernaea lophiara* (2.50%). It has been reported that lernaeid copepods are the most lethal parasites, especially in young fish where only a few specimens can kill the fish (Kabata, 1985; Khalifa and Post, 1976; Bauer *et al*., 1973).

According to Tufail *et al*. (2014) growth performance of *Catla catla* was better in June because of low parasitic load. The parasitic load increased in the months of July and August that may have reduced the growth performance of *Catla catla* that was contradictory to our study. Marcogliese (1991) and Medeiros and Maltchik (1999) also observed similar effect that prevalence of *Lernaea* on fish typically increases during the summer when water temperatures exceed 25°C, although

parasitized fish can be found during the fall and winter as reported by Bulow *et al*. (1979). Tufail *et al*. (2012) further suggested that *Lernaea cyprinacea* infestation and ulceration percentage was low in the month of June, gradually increased in July to September with increased ulceration percentage. However, treatment of *Lernaea cyprinacea* with potassium permanganate (prophylactic management of disease) decreased infestation but percentage of ulceration remained high. Joy and Jones (1973) observed similar ulceration on a serranid fish where *Lernaea cruciata* infestation was observed accompanied by an inflammatory response characterized by an extensive proliferation of fibrous connective tissue elements, wherein, both the dermis and musculature of the host were involved.

Iqbal *et al*. (2012) reported three species of *Lernaea*; *Lernaea cyprinacea, Lernaea polymorpha* and *Lernaea oryzophila* on *Labeo rohita* (prevalence 98.69%), Hussain (1998) studied five species (*Lernaea. Polymorpha, Lernaea arcuata, Lernaea lophiara. Lernaea cyprinacea, Lernaea oryzophila)* and Tasawar *et al*. (1999) reported 4 species which was inconsisten with our observations where only single species was observed. This difference in species prevalence may be due to quality of water examined and type of environment studied. However, *Lernaea cyprinacea* dominated in term of number than other *Lernaea* species in all hosts. The prevalence of *Lernaea cyprinacea* in *Labeo rohita, Catla catla* and *Ctenopharyngodon idella* recorded highest may be related to their trophic behaviors. Bauer *et al*. (1973) observed higher infestation in the *Ctenopharyngodon idella* than *Aristichthys nobilis* and *Hypophthalmichthys molitrix*. Our results are comparable to Tasawar *et al*. (1999). Iqbal *et al*. (2001b,c) and Tasawar *et al.* (2007a,b) reported the highest prevalence of *Lernaea cyprinacea* in *Catla catla*. These studies therefore suggest that *Catla catla* is the most susceptible host of *Lernaea cyprinacea*.

Iqbal *et al*. (2001c, 2012) and Tasawar *et al.* (1999) observed highest prevalence of the parasite in the late winter December to April (water temperature 13-23ºC) and low during the summer months. Maximum intensity of infection reached 9 specimens per fish (*Catla catla*) which is similar to Ho and Kim (1997) findings in European fingerling. Adult specimens attached the skin, primarily the ventral part of the body (mainly bases of the pectoral and pelvic fins). Acute inflammation, necrosis and capsule (with collagen fibers) formation were the typical characteristics of infection (Ho and Kim, 1997). Abdomen and ventral side of the body of the fish seems the most common site of attachment of *Lernaea* species. It is convincing that *Lernaea* have definite affinity for abdomen and at the base of fins. These sites offer more protection in water and further these may be more easily penetrated by the parasite. This support the hypothesis proposed by Medeiros and Maltchik (1999) that *Lernaea cyprinacea* prefers that location which offers greater protection against water current. However, this particular way of attachment of *Lernaea* is very pathogenic by virtue of nature of its attack (Khalifah and Post, 1976; Kabata, 1985; Sharif and Roberts, 1989).

Treatment of *Lernaea cyprinacea* includes baths, dips and pond treatment. First two are time consuming, laborius, stress causing and less effective than that of chemical treatment of pond. In Punjab, incidencs of mass fish mortalities due *Lernaea* infestation are rare but chronic and long term effects are very common which severely affects the potential of fish production unit (Shariff and Roberts, 1989; Piasecki *et al*., 2004; Iqbal *et al*., 2001). Our studies were in agreement with that of Iqbal *et al*. (2012) who used different concentration of thunder (0.10-0.20 ppm) in carp culture and suggested 0.20 ppm as most effective concentration without causing any harm to fish and pond flora and fauna. Infective stages of parasite died after repeated applications of chemical and further minimized chances of new infection and reinfection. Abidi *et al*. (2000) and Ayub *et al*. (2000) also recommended 0.5 to 1.5 ppm concentration of thunder for effective eradication of *Lernaea cyprinacea.* Organophosphate trichlorophan (Kashara, 1962), formalin (Putz and Bowen, 1964), Lexone (Gopalkrishan, 1964) and Benzene Hexachloride (Hoffman and Mayer, 1974) also listed as effective chemicals for *Lernaea cyprinacea* control. Masoten spraying at 0.2-0.5 ppm concentration twice a month in summer also recommended but with precautionary measures (Kabata, 1985). Kabata

(1985) used Dipterex with every three days of repetition in Indonesia and Trichlorophon in China with 24 h of repetition to eliminate the parasite. Burtle and Morison (1987) suggested Dimilin (10 ppb) in ponds of golden shiner to eradicate *Lernaea* and other copepods. 'Thunder'at 0.25 ppm in our study is in accordance with the Minhas *et al*. (2001), Iqbal *et al*. (2012), Abidi *et al*. (2000) and Ayub *et al*. (2000). Hemaprasanth *et al.* (2008) used intramuscular administration of Doramectin (200 bbp/kg of fish weight) of heavily infected adults and brood stocks of carps infected with *Lernaea cyprinace*. Thunder is biologically safe as it is biodegradable and degenerated after 36 h without causing any negative effect on the water quality parameters and other water fuana and flora (Abidi *et al*., 2000). Treatment of *Lernaea cyprinace* with 'Thunder' (0.10-0.25ppm) gives promising results without adverse effect on fish life. These findings have proved that *Catla catla* is the most susceptible fish to *Lernaea cyprinacea* infestation and its appropriate control for conservation of this precious and declining fish is of extreme importance. Though DDVP worked well in its control but its heavy infestation really weaken fish incapacitating its feeding and competing abilities with its counterparts. Reasons of this very low resistance to parasitic infestation could be many however further research is in progress to investigate that what attracts parasite to this fish and what are the reasons that it cannot defend it from enemenity of this menace.

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