Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Lernaeid parasites prevalence in commercial freshwater fish species at various fish farms in Pakistan

Prevalência de parasitas de *Lernaea* em espécies comerciais de peixes de água doce em várias pisciculturas no Paquistão

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Abstract

Reports abound on *Lernaea* parasitizing the brood stock, fingerlings, and marketable-sized culturable freshwater fish species in various parts of the world. We investigated seven small-scale aquaculture farms and how the prevailing *Lernaea* is impacting them. Randomly seven fish farms were selected to determine the prevalence percentage of lernaeid ectoparasites. Relevant information of the fishponds to estimate the various aspects such as effects of water source and quality, feed, stocking density, treatment used, and weight and length of fish, concerned with *Lernaea* infestation and prevalence was gathered. The results indicated that *Catla catla* (F. Hamilton, 1822) showed highest prevalence (41.7%) among the seven fish species, whereas *Oreochromis niloticus* showed zero. Other five fish species *Ctenopharyngodon idella, Cirrhinus cirrhosis, Cyprinus carpio, Labeo rohita* and *Hypophthalmichthys molitrix* showed 13.2%, 8.1%, 7.7%, 7.4%, 0.9% prevalence, respectively. In Royal Fish Farm 84.3% lernaeid infestation was observed, while no parasite was observed in the Vicent's Chunnian fish farm. The water source, quality, feed, fertilizers, stocking density, water temperature, and potential treatment options displayed varying tendencies among fish farms and prevalence. Depending on the weight and length, the highest prevalence (56.7%, and 66.7%) was observed in 3501-4000 g and 81-90 cm groups. The infestation rate varied in various fish body parts with the dorsal fin the most vulnerable organ and showed 2.3% overall prevalence (while 18.4% contribution within total 12.6% infestation). Out of 147 infected fish samples, 45 were extensively contaminated by *Lernaea* spread. In conclusion, our findings confirm that *Lernaea* could pose a considerable threat to marketable fish, and various treatment options should be educated to the farmers to help mitigate the spread and potential losses. Furthermore, *Catla catla* is more vulnerable to *Lernaea* infestation (41.7%), so are the fish species being cultured at higher stocking densities.

Keywords: *C. catla*, infestation, *Lernaea*, pond aquaculture, prevalence.

Resumo

Abundam os relatórios sobre *Lernaea* parasitando o estoque de cria, alevinos e espécies de peixes de água doce cultiváveis de tamanho comercial em várias partes do mundo. Investigamos sete fazendas de aquicultura de pequena escala e de que maneira a *Lernaea* predominante está impactando-as. Aleatoriamente, sete fazendas de peixes foram selecionadas para determinar a porcentagem de prevalência de ectoparasitas de *Lernaea*. Foram recolhidas informações relevantes sobre os viveiros de peixes para estimar os vários aspectos, tais como efeitos da fonte e qualidade da água, alimentação, densidade de povoamento, tratamento utilizado e peso e comprimento dos peixes, relacionados com a infestação e prevalência de *Lernaea*. Os resultados indicaram que *Catla catla* (F. Hamilton, 1822) apresentou maior prevalência (41,7%) entre as sete espécies de peixes, enquanto *Oreochromis niloticus* apresentou zero. Outras cinco espécies de peixes *Ctenopharyngodon idella*, *Cirrhinus cirrhosis*, *Cyprinus carpio*, *Labeo rohita* e *Hypophthalmichthys molitrix* apresentaram 13,2%, 8,1%, 7,7%, 7,4%, 0,9% de prevalência, respectivamente. Em Royal Fish Farm, 84,3% de infestação de *Lernaea* foi observada, enquanto não se observou nenhum parasita na fazenda de peixes Chunnian de Vicent. A fonte de água, qualidade, ração, fertilizantes, densidade de estocagem, temperatura da água e opções de tratamento potenciais exibiram tendências variadas entre as fazendas de peixes e prevalência. Dependendo do peso e comprimento, a maior prevalência (56,7% e 66,7%) foi observada nos grupos

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Received: July 2, 2021 – Accepted: August 13, 2021

de 3501-4000 g e 81-90 cm. A taxa de infestação variou em várias partes do corpo dos peixes, sendo a nadadeira dorsal o órgão mais vulnerável e apresentou 2,3% de prevalência geral (enquanto 18,4% de contribuição dentro do total de 12,6% de infestação). Das 147 amostras de peixes infectados, 45 estavam amplamente contaminadas pela propagação de *Lernaea*. Em conclusão, nossos resultados confirmam que *Lernaea* pode representar uma ameaça considerável para peixes comercializáveis, e várias opções de tratamento devem ser educadas para os agricultores para ajudar a mitigar a propagação e as perdas potenciais. Além disso, *Catla catla* é mais vulnerável à infestação por *Lernaea* (41,7%), assim como as espécies de peixes sendo cultivadas em densidades de estocagem mais altas.

Palavras-chave: *C. catla*, infestação, *Lernaea*, aquicultura em lago, prevalência.

1. Introduction

Aquaculture has emerged as the fastest growing food production sector as well as it is offering reliable alternatives to high quality production of protein (Abbas et al., 2023; Haider et al., 2018; Iqbal et al., 2020a; Jewel et al., 2020). However, it is constantly facing several emerging hazards that are threatening to the sustainable aquaculture production (Batool et al., 2018; Iqbal et al., 2020b; Mehboob et al., 2017; Ray et al., 2021). The pervasiveness of ectoparasites is a significant hazard to the rapidly proliferating aquaculture industry and with its subsequent step at the intensive farming at higher stocking densities. Intensive and semi-intensive culture methods increase the infestation risks by ectoparasites that directly damage the fish body and jeopardize it to the attack of secondary pathogens to invade and cause heavy economic losses (Piasecki et al., 2004), leading to decreased consumer preference and subsequently increased market losses (Iqbal et al., 2020c). One of the most common and dangerous families of ectoparasites of cultured fish species is *Lernaeidae* (anchor worms), usually associated with parasitism of freshwater fishes.

Among *Lernaeidae* family of copepods, *Lernaea cyprineace* (Linnaeus, 1758) is the most frequently occurring and proportionately more dangerous than other sister species. Lernaeid copepods affect fish gills, fins, and body tissues and induce necrosis and disruption. When a female is attached to any body part of fish or specially gills, it leads to severe inflammation, muscular necrosis, and haemorrhage, which paves the way for secondary infections by bacterial and fungal pathogens (Berry Junior et al., 1991; Khalifa and Post, 1976; Lester and Hayward, 2006). *Lernaea* parasites are discovered everywhere in freshwater and infect several freshwater fish species. They have a high degree of intraspecific morphological variability and interspecific similarities; hence their classification has remained challenging for freshwater fish pathologists.

Some decades ago, studies were conducted to investigate the anchors of *Lernaea* and are an excellent tool to classify various *Lernaea* species. However, it is not suitable to entirely rely on the anchors during the classification of these ectoparasites (Hua et al., 2019). Pathogenic *Lernaeidae* family members cause disease in freshwater fishes worldwide and imply dangerous health effects to infected hosts (Lester and Hayward, 2006). An investigation declared that this parasite has originated from Asia and spread through several regions of the world by the locomotion of farming species by clinging with anchor's help (Innal and Avenant-Oldewage, 2012). This parasite has now been reported worldwide, from Eastern Australia, Asia, Southern Africa, and Europe (Hoffman, 2019; Lester and Hayward, 2006). It has been observed that fish fingerling mortality is frequently happening due to these parasites' infestation on a global scale. Therefore, it is imperative to study its prevalence, considering the heavy losses incurred (Hemaprasanth et al., 2008).

Previous research has reported that *Lernaea cyprinacea* (*L. cyprinacea*) infection has emerged as a significant threat to South Asia's carps culture. It has been recurrently reported from *Cirrhinus mrigala* (F. Hamilton, 1822), *Hypophthalmichthys molitrix* (Valenciennes, 1844), *Catla catla* (F. Hamilton, 1822), *Labeo rohita* (F. Hamilton, 1822), *Ctenopharyngodon idella* (Valenciennes, 1844), *and Labeo fimbriatus* (Bloch, 1795) with variable intensity (Iqbal et al., 2001a; Nandeesha et al., 1984; Nandeesha et al., 1985; Tamuli and Shanbhogue, 1996). However, it has not been reported on *Cyprinus carpio* (Linnaeus, 1758) and *Labeo calbasu* (F. Hamilton, 1822) from the same geographical region. Later, Hemaprasanth et al. (2011) first reported the *L. cyprinacea* infestation in the *C. carpio* reared in the polyculture system.

District Kasur of Punjab, Pakistan, is a vital fish farming zone as the Ravi River bounds it in the north-west and river Sutlej in the south-east along the bordering region of India (Atique et al., 2020). Simultaneously, Beas River's old course bifurcates the district into two equal parts locally known as Hither and Uthar or Mithan Majh. It is considered the primary fish market in Punjab province, competing for Lahore, Rawalpindi, Gujranwala, Faisalabad, Multan, and Sahiwal. Apart from valuable catch fisheries, several fish farms have been developed to boost this economically vital region's aquaculture potential. Head Baloki, Changa Manga, Chunian, and the University of Veterinary and Animal Sciences (UVAS) fish farms are some of the most crucial aquaculture hotspots promoting. Recently, local fish farmers have reported *Lernaea* infestation's prevalence in their small-scale to large-scale fish farms.

Therefore, considering the importance of the Kasur region, we planned this study to investigate the *Lernaea* infestation and prevalence from various fish farms of private and public proprietorship. We aimed at investigating the presence, load, and prevalence of *Lernaea* in seven commercially important aquaculture fish species in Pakistan.

2. Material and Methods

2.1. Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received from Ethical Review Committee, Office of Research, Innovation & Commercialization, University of Veterinary and Animal Sciences, Lahore, Pakistan. The given methods of Restraint, Handling and Protocols for Lab Animals were followed.

2.2. Study area and fish sampling for Lernaea prevalence

Seven randomly selected public and privately-owned fish farms in district Kasur, Punjab, Pakistan were targeted for the study (see Figure 1). A questionnaire was prepared to collect the intended information about fish farming's various aspects by directly investigating the fish farms' owners/managers. Fish samples from the surveyed fish farms using seine net and cast/throw nets on a bi-monthly basis from August 2019 to January 2020 were collected. Total 1164 fish samples were examined, including 323 of Rohu (*L. rohita*), 422 Grass carp (*C. idella*), 37 Mori (*C. cirrhosis*), 144 Thaila (*C. catla*), 107 Silver carp (*H. molitrix*), 105 Tilapia (*Oreochromis niloticus*, Linnaeus, 1758) and 26 of common carp *(C. carpio)* for potential *Lernaea* infestation and prevalence. The fish body weight and length were also measured. *Lernaea* were observed on different body organs and the fish body to check parasite's organ specificity, including lateral, dorsal, ventral, caudal, pectoral fin, anal region, gills, eyes, mouth, and snout of the fish. Using forceps and $KMnO_4$ solution for easy detachment *Lernaea* parasites were removed from the commercially harvested fish and preserved in the centrifuge tubes using 10% formalin and 95% ethanol solutions for further identification at the laboratory.

2.3. Morphology of Lernaea

Lernaea samples isolated from fish were brought to the fish diagnostic and health management lab of Fisheries and Aquaculture Department, UVAS, Ravi Campus, Pattoki for species identification. The identification key of Kabata (1985), Margolis and Kabata (1988) and Monod (1932) were followed.

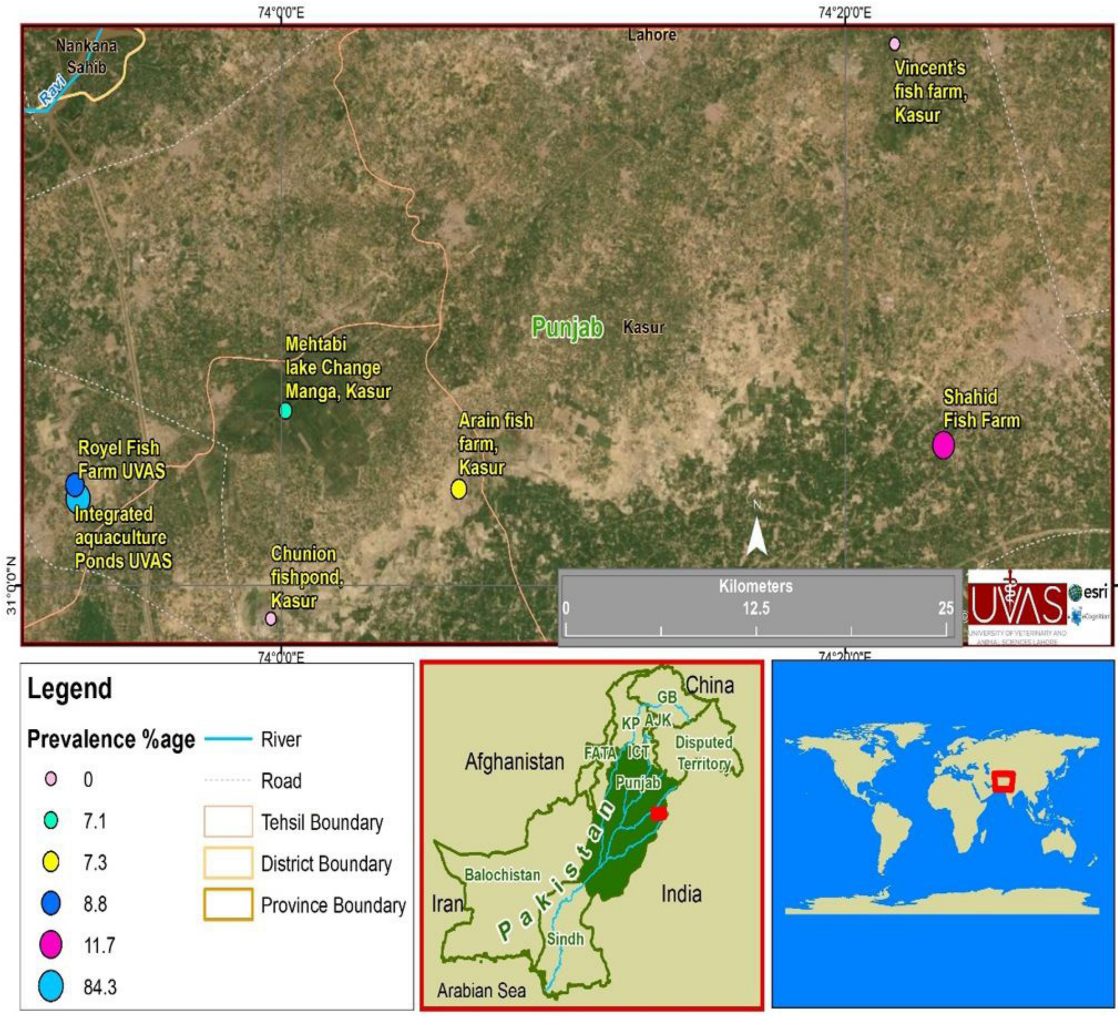

Figure 1. Map showing localities studied during this research trial.

2.4. Statistical analysis

For the statistical analysis, the data was checked for its normality before treating for statistical evaluations. The obtained dataset was analyzed with the help of statistical package for social sciences (SPSS, v. 21). To compare the various farms and fish species, we used the Chi-square test. The obtained results were presented in tables.

3. Results

The data from seven fish farms showed that most of the time all the fish farmers used underground water pumped out with tube-well water for aquaculture. However, some of the farms also relied upon the combination of tube-well, rain, and canal water as the water resources (Chunnian and Shahid fish farms). These fish farms displayed varying prevalence tendencies of *Lernaea* (as shown in Table 1). Farms using water from sources other than the tube-well indicated lower *Lernaea* prevalence than the farms using tube-well water only. Similarly, farms that used manure and other fertilizers showed zero prevalence than the farms utilizing urea or diammonium phosphate (DAP) for pond fertilization. Treatments used to disinfect ponds before culturing aquaculture species and during pond management also influence disease rate and *lernaea* prevalence (as shown in Table 1).

Most fish farmers cultured Indian major carps and Chinese carps except for Chunian Fish Farm that stocked tilapia. The farm area differed significantly; however, the stocking density did not indicate dense fish stocking signs that could cause higher chances of parasitic outbreaks. Stocking density with respect to the pond area also showed an essential connection to the *Lernaea* infestation. Farms with higher stocking density showed a heavy *Lernaea* infestation, whereas applications of lime reduced the infestation. It was also noticed that pond liming was the best treatment option for *Lernaea* control. The selected water

Table 1. Farm and feeding details along with the potential infestation and treatment methods of *Lernaea* at the seven fish farms surveyed during the study.

Fish Farm	Size	Water source	Feed	% Feed given	Fertilizer	Stocking density/ acre	Fish Species	*Lernaea* infestation	Treatment used
Royal Fish Farm, UVAS, Pattoki	2 acres	Tube well	AMG Fish Feed 22% CP	2%	Manure, Urea	1000	Rahu, Mori, Thella, Silver, Grass	84.3	Liming, $KMnO_4$
Integrated Aquaculture Fish Farms, UVAS, Pattoki	1 acre	Tube well	Supreme Feed (25% CP)	2%	Duck droppings, Urea	800	Rahu, Mori, Grass carp	8.8	Organophosphates
Mehtabi Lake Change Manga	8 acres	Tube well	Rice polish, Wheat bran, Soya been	2%	DAP, Urea	2000	Rahu, Mori, Thella, Grass carp, Silver, Hybrid (Rahu + Thella)	7.1	Liming, $KMnO_4$
Vincent's Fish Farm	3 acres	Tube well	Forage / Fodder	2-4%	DAP, Urea, Manure	800	Grass carp, Rahu, Thella	0	Liming
Arain Fish Farm, Kasur	4 acres	Tube well	Rice police	1.5-2.5%	DAP, Urea, Manure	1000	Rahu, Thella, Mori, Grass carp, Common carp	7.3	Liming
Chunian Fish Farm, Kasur	1 acre	Rain, Canal water, Tube well	Forage, Husk, Cow dung, Fertilizer, Rice polish	2-4%	Manure	1200	Tilapia	0	Liming
Shahid Fish Farm, Kasur	2 acres	Tube well, Canal water	Rice polish, Maize gluten, Soya been	2%	DAP, Urea, Manure	1000	Rahu, Thella, Mori, Grass carp	11.7	No treatment measures taken

quality parameters were observed within the acceptable ranges for aquaculture practices (as shown in Table 2).

A total of 1164 samples of freshwater fish species were examined from seven fish farms, among which only 147 (12.6%) samples were observed with the *Lernaea* infestation. Total 12 species of *Lernaea,* that included *L. cyprinacae*, *L. cruciata*, *L. polymorpha*, *L. ctenopharyngodanis*, *L. devastatrix*, *L. lophiara*, *L. multilobosa*, *L. temnocephala*, *L. esocina*, *L. barnimiana*, and two unidentified species of *Lernaea* due to potential sample damages or difficulties in identification were observed in this study (see Figure 2a-l). The most common species observed in this study was the *L. cyprinacea.* The various body organs of fish for possible anchoring of the parasites were observed, and the details are shown in Figure 3. It shows the hotspots of *Lernaea* on the whole fish body, head and snout, and around the eye.

The highest *Lernaea* infestation was observed at the Royal fish farm (84.3%), Integrated Aquaculture Fish Farm, UVAS, Pattoki (8.8%), whereas the lowest (no infestation) was observed at the Vicent's and Chunian fish farm (as shown in Table 3). Among the seven fish species sampled during the study, the highest prevalence of *Lernaea* was observed on *C. catla* (41.7%), *C. idella* (13.2%), *C. mrigala* (8.1%), *C. carpio* (7.7%), *and L. rohita* (7.4%). In contrast, no *Lernaea* infestation was observed on *O. niloticus* (as shown in Table 4).

This study explored the most vulnerable weight and length stages of cultured fish species to the *Lernaea* infestation. The results concluded that *Lernaea* had a different prevalence in the various age groups (as shown in Table 5). For instance, *C. mrigala* and *C. carpio* showed higher prevalence in lower body weight groups, while *C. catla, C. idella* and *H. molitrix* showed higher prevalence at older stages or higher weight groups (3000 – 4000g). The highest prevalence (56.7% and 66.7%) was observed in the 3501-4000 g and 81-90 cm groups, respectively. For *L. rohita, C. idella, C. mrigala, H. molitrix* and *C. carpio,* the highest prevalence was recorded as 75.0%, 29.7%, 50.0%, 25.0% and 50.0% within 3501-4000 g, 501-1000 g, ≤500 g, 3501-4000 g and ≤500 g weight groups, respectively.

Table 2. Records of the selected water quality parameters and vegetation types at the surveyed fish farms.

Fish farm	Vegetation	WT (°C)	pH	DO (mg/L)	BOD	TSS (mg/L)	Salinity (%)
Royal Fish Farm, UVAS, Pattoki	Grasses, Weeds, Algae, Fungi	14±19	8.9±1.0	7.5±1.4	5.8±0.2	10.0±0.3	10.0±0.2
Integrated Aquaculture Ponds UVAS, Pattoki	Algae, Grasses	15±15	7.5±1.5	7.1±0.4	4.2±0.1	8.0±0.7	6.5±0.4
Mehtabi Lake, Change Manga	Algae	23±10	7.3±1.1	6.3±0.7	3.9±0.1	7.1±0.9	7.9±0.3
Vincent's Fish Farm, Kasur	Algae, Weeds	15±14	6.9±1.3	7.2±0.7	3.0±.3	4.0±1.3	4.5±0.5
Arain Fish Farm, Kasur	Grasses, Algae	19±15	7.1±0.9	6.4±1.4	3.7±0.1	5.5±0.9	5.6±0.9
Chunian Fish Farm, Kasur	Algae	12±16	7.0±1.5	6.5±05	2.9±0.4	3.2±0.6	4.0±0.1
Shahid Fish Farm, Kasur	Grasses, Algae, Weeds	16±15	8.7±1.3	6.8±0.6	3.5±0.3	6.0±0.9	8.3±0.8

WT = water temperature; DO = Dissolved oxygen; BOD = biological, oxygen demand; TSS = Total suspended solids.

Table 3. Percentage prevalence of *Lernaea* at surveyed fish farms.

Fish Farm	Samples	Samples infected	% Prevalence
Royal Fish Farm, UVAS, Pattoki	89	75	84.3
Integrated Aquaculture Ponds UVAS, Pattoki	57	5	8.8
Mehtabi Lake, Change Manga	70	5	7.1
Vincent's Fish Farm, Kasur	47	0	0
Arain Fish Farm, Kasur	702	51	7.3
Chunian Fish Farm, Kasur	105	0	0
Shahid Fish Farm, Kasur	94	11	11.7
Total	**1164**	**147**	**12.6**

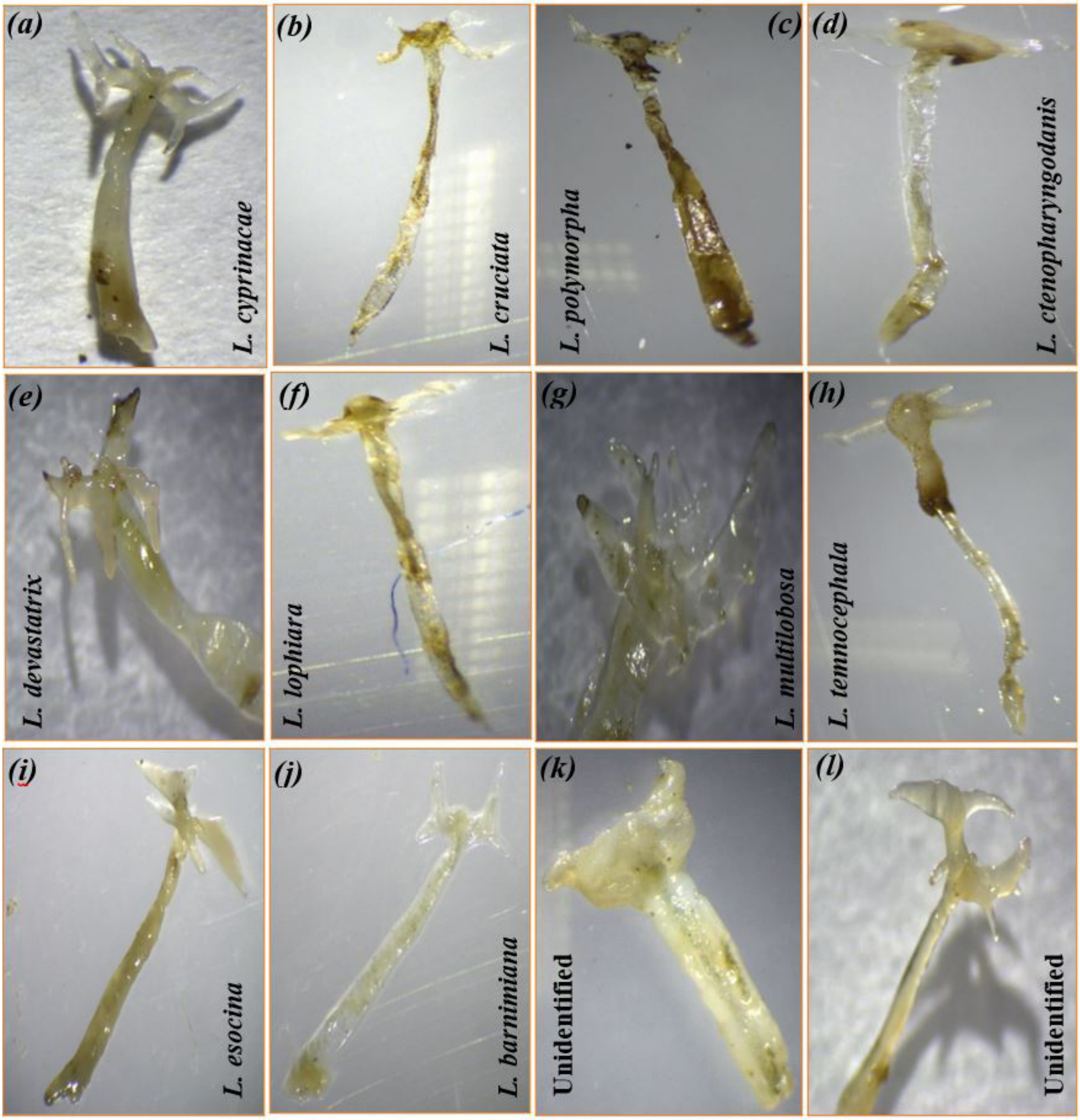

Figure 2. Snapshot of prevalent *Lernaea* species identified (a-j) and unidentified (k-l) during the survey of various fish farms.

Table 4. *Lernaea* prevalence with respect to fish species cultured at fish farms.

Fish species	Examined Samples	Infected Samples	Prevalence (%)
Labeo rohita	323	24	7.4
Ctenopharyngodon idella	422	58	13.2
Cirrhinus cirrhosis	37	3	8.1
Catla catla	144	60	41.7
Hypophthalmichthys molitrix	107	1	0.9
Cyprinus carpio	26	2	7.7
Oreochromis niloticus	105	0	0
Total	**1164**	**147**	**12.6**

Table 5. Percentage prevalence of *Lernaea* with respect to weight and length of observed fish species.

Weight and Length categories	*L. rohita*	*C. idella*	*C. cirrhosis*	*C. catla*	*H. molitrix*	*C. carpio*	Total prevalence (%)
≤500g	7.1%	27.8%	50%	16.7%	0%	50%	13.3%
10-20cm	0%	7%	-	16.7%	0%	-	2.9%
501-1000g	4.2%	29.7%	9.1%	5.9%	0%	0%	12.7%
21-30cm	11.1%	41.5%	50%	9.1%	0%	50%	20%
1001-1500g	0%	5.2%	6.7%	0%	0%	-	2.6%
31-40cm	0.8%	28.8%	6.7%	0%	0%	0%	8.1%
1501-2000g	2.7%	0%	0%	0%	0%	0%	0.6%
41-50cm	1.8%	2.9%	9.1%	6.7%	-	-	2.9%
2001-2500g	22.2%	0%	0%	33.3%	0%	5.3%	8.9%
51-60cm	13.3%	1.4%	0%	29.4%	0%	0%	8.4%
2501-3000g	60%	4.1%	-	63.3%	0%	0%	30%
61-70cm	50%	50%	0%	85%	0%	5.9%	25.2%
3001-3500g	42.9%	12%	-	84.6%	0%	-	39.4%
71-80cm	57.1%	21.2%	-	76.5%	20%	0%	39.7%
3501-4000g	75%	38.5%	-	88.9%	25%	-	56.7%
81-90cm	66.7%	-	-	-	-	-	66.7%
Total	7.4%	13.7%	8.1%	41.7%	0.9%	7.7%	

The relationship between length and infestations of *Lernaea* showed that the highest prevalence was 66.7%, 50.0%, 50.0%, 20.0%, and 50.0% within 71-80 cm, 61-70 cm, 21-30 cm, 71-80 cm, and 21-30 cm length groups for *L. rohita, C. idella, C. cirrhosis, H. molitrix* and *C. carpio,* respectively (as shown in Table 5).

The fish body was distributed into 12 zones and examined for potential hotspots of *Lernaea* infestation (see Figure 3). It was observed that the dorsal fin was the most vulnerable part of the fish body and showed 2.3% overall prevalence (while 18.4% contribution within total 12.6% infestation). The highest prevalence was recorded for different species within different body part was 0.5%, 1.3%, 0.2% 0.9% and 0.1% in *L. rohita*, *C. idella*, *C. cirrhosis*, *C. catla,* and *H. molitrix*, regarding dorsal fin for the first three species and the fifth one but ventrodorsal and lateral body region for *C. catla.* Among the samples, 45 (30.6%) were infected with all body parts, especially in *C. catla* (32 pieces), with an infestation on the eyes and head region. One *L. rohita* was infested on its snout region, while another sample of *C. idella* was infested on the head region (as shown in Table 6).

The fish sampling was performed bi-monthly (Aug-Sep, Oct-Nov, and Dec-Jan), and the prevalence during these periods are presented in Table 7. The highest infestation was 13.6% observed during Dec-Jan, while 8.3% and 3.3% were recorded during Aug-Sep and Oct-Nov, respectively. The individual percentage of infestation also recorded for different species studied during these periods. The highest prevalence was observed on *L. rohita* (5%) during Aug-Sep and for *C. idella* (14.5%), *C. cirrhosis* (9.4%), *C. catla* (45.8%), *H. molitrix* (1%), and *C. carpio* (7.7%) were recorded during Dec-Jan.

4. Discussion

Lernaeidae occupies a unique position owing to their extraordinary pathogenic effect and financial damages in fish farming. Specifically, the *L. cyprinacea* (Linnaeus, 1758) is the primary causative agent of lernaeaosis in Indian Major carps and Chinese carps and turns the saleable fisheries products nonsalable because it inflicts severe tegumentary lesions (Tavares-Dias and Martins, 2017). Furthermore, such parasitic diseases can demote reproductive ability and negatively influence feed conversion efficiency in fish, causing a decline in fish growth and cultured fish's overall performance. The present study aimed at investigating the *Lernaea* prevalence in commercial and public fish farms in district Kasur, Pakistan. We collected the relevant data from the farm owners and managers about pond inputs, water quality, overall pond status, *Lernaea* prevalence, and potential strategies to control it by using a questionnaire. The *Lernaea* samples collected from the fish species and their identification indicated ten known species viz. *L. cyprinacea, Lernaea polymorpha* (Yü, 1938), *Lernaea cruciata* (Lesueur, 1824), *Lernaea ctenopharyngodanis* (Yin, 1961), *Lernaea devastatrix* (Boxshall, Montú & Schwarzbold, 1997), *Lernaea lophiara* (Harding, 1950), *Lernaea multilobosa* (Jafri and Mahar, 2003), *Lernaea temnocephala* (Cunnington, 1914), *Lernaea esocina* (Burmeister, 1835), and *Lernaea barnimiana* (Hartmann 1865). However, we also recorded two unknown species owing to difficulties in species identification based on morphological characteristics. The other reasons could also include the damage of samples during *Lernaea* removal from the fish skin and organs. Iqbal et al. (2012) have reported *L. cyprinacea, L. polymorpha* and *Lernaea oryzophila* (Monod, 1932) on *L. rohita* and *C. idella*. *L. ctenopharyngodonis* and some other

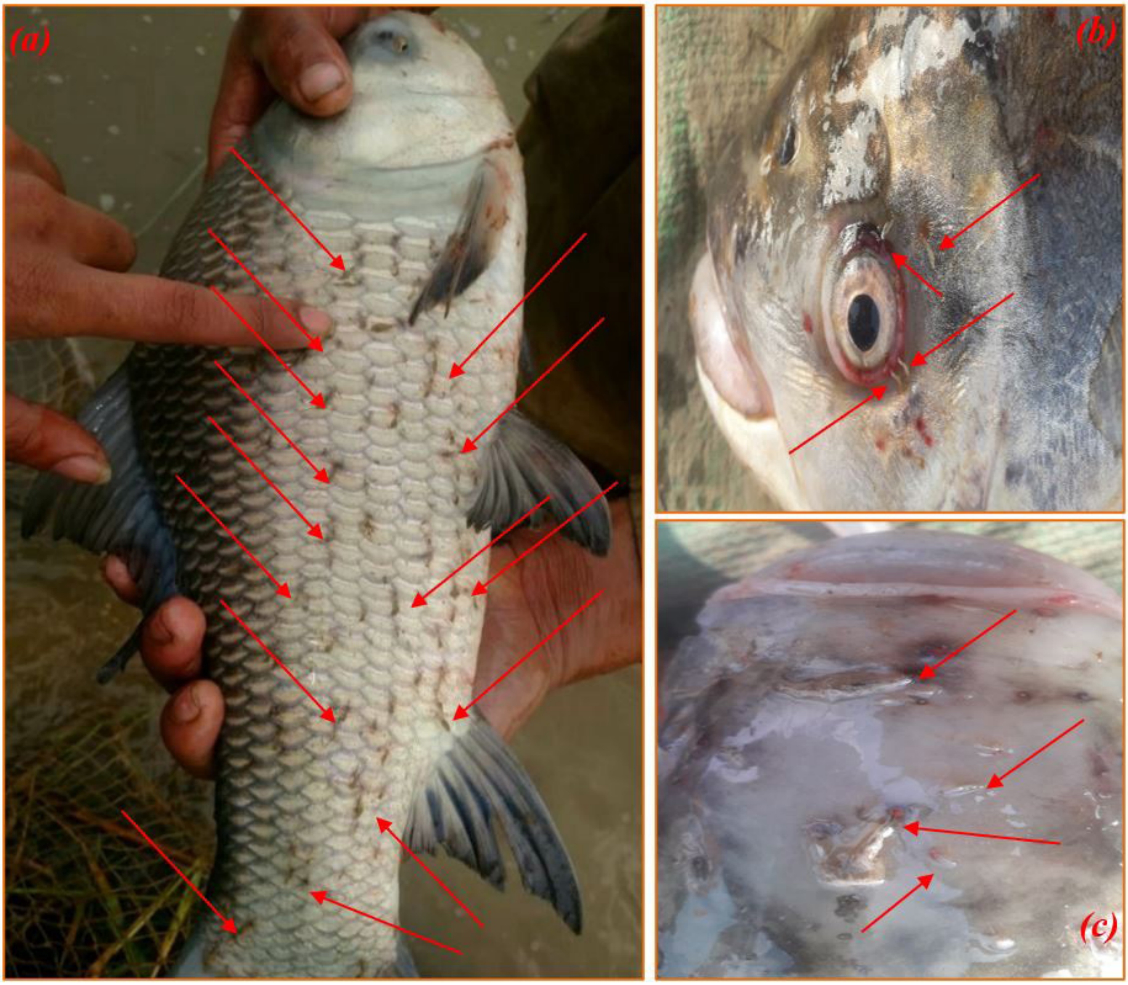

Figure 3. Hotspots of *Lernaea* on fish body (a), around the eye (b) and snout (c), identified during the survey.

unknown species of *Lernaea* from the University of the Punjab research fish farms. The *L. cyprinacea* was the most prevalent and dominant. Outcomes of Hemaprasanth et al. (2008) and Tasawar et al. (2007) also corroborated our findings. The *Lernaea* species are cosmopolitan parasites of several freshwater fish species reported from all the countries famous for fish culture. However, their classification and identification are controversial due to morphological intraspecific variability and interspecific similarities (Hua et al., 2019).

The fish samples collected from various aquaculture ponds showed that ponds fed with tube well for their freshwater supply were the most affected. Among all specimens examined, the highest prevalence was observed in *C. catla* (41.7%). Tasawar et al. (2007) reported 96 out of 120 *C. catla* fish samples as infested, showing an overall prevalence of 80%. Abbas et al. (2014) presented that not a single lernaeid ectoparasite specimen was observed on *C. carpio,* and it emerged as the most resistant fish species among Chinese and Indian major carps. However, our results report 7.7% *Lernaea* prevalence in *C. carpio*. Rahnama et al. (2016) studied 10.7% prevalence of *Lernaea* species reported from 107 infected *C. carpio* samples out of 1000, while a 30.1% prevalence was calculated by Sayyadzadeh et al. (2016), with these studies in close approximation with our research. Piasecki et al. (2004) reported *C. carpio* as the best-known host for lernaeid species. Heavy *Lernaea* infestation in *C. catla* recorded during the current study could be due to considerable fish body weight, slow movement, feeding preferences in the pond where *Lernaea* growth conditions were in an optimum range. Such energy crises result in lethargy and immune deficiency in fish that make them more vulnerable to *Lernaea* infestation and secondary infections. These findings suggest that a species could be non-affected in one part, but the same species could be highly infected in other parts of the world, alluding to infestation uncertainty.

The maximum prevalence at the dorsal fin followed by the caudal fin was observed, which corroborates several previous findings and indicates the most favorite places for the anchoring parasites (Iqbal et al., 2012; Innal et al., 2017; Mirzaei, 2015; Stavrescu-Bedivan et al., 2014).

Table 6. Prevalence (%) of *Lernaea* with respect to different body parts in different fish species observed.

Body part	*L. rohita* (%)	*C. idella* (%)	*C. cirrhosis* (%)	*C. catla* (%)	*H. molitrix* (%)	*C. carpio* (%)	*Total (%)*	*% Of 12.6% infestation*
Lateral line	5 (0.4)	1 (0.1)	1 (0.1)	1 (0.1)	-	-	8 (0.7)	5.4
Dorsal fin	6 (0.5)	15 (1.3)	2 (0.2)	3 (0.3)	1 (0.1)	-	27 (2.3)	18.4
Ventral body plane	-	5 (0.4)	-	2 (0.2)	-	-	7 (0.6)	4.8
Ventral, dorsal and lateral body plane	4 (0.3)	9 (0.8)	-	11 (0.9)	-	1 (0.1)	25 (2.1)	17
Caudal region	1 (0.1)	10 (0.9)	-	2 (0.2)	-	1 (0.1)	14 (1.2)	9.5
Head	-	1 (0.1)	-	-	-	-	1 (0.1)	0.7
Pectoral fin	1 (0.1)	4 (0.3)	-	1 (0.1)	-	-	6 (0.5)	4.1
Pelvic fin	-	4 (0.4)	-	-	-	-	5 (0.4)	3.4
Snout	1 (0.1)	-	-	-	-	-	1 (0.1)	0.7
Whole fish	5 (0.4)	8 (0.7)	-	32 (2.7)	-	-	45 (3.9)	30.6
Eyes	-	0	-	5 (0.4)	-	-	5 (0.4)	3.4
Mouth	-	0	-	3 (0.3)	-	-	3 (0.3)	2
Total	2.1%	5%	0.3%	5.2%	0.1%	0.2%	147 (12.6)	-
% Within 12.6% infestation	15.6	39.5	2	40.8	0.7	1.4	-	100

Table 7. Percentage prevalence of *Lernaea* within fish species with respect to study periods.

Study Period	Fish species	Examined samples	Infected samples	Prevalence %	Percentage within 12.6 prevalence
Aug-Sep.					
	Rahu,	21	1	4.8%	
	Grass carp	15	2	13.3%	
	Total	36	3	8.3%	2%
Oct-Nov.					
	Rahu	40	2	5%	
	Grass carp	27	1	3.7%	
	Mrigal carp	5	0	0%	
	Thella	13	0	0%	
	Silver	5	0	0%	
	Total	90	3	3.3%	2%
Dec-Jan.					
	Rahu	262	21	8%	
	Grass carp	380	55	14.5%	
	Mrigal carp	32	3	9.4%	
	Thella fish	131	60	45.8%	
	Silver carp	1021	1	1%	
	Tilapia	105	0	0%	
	Common carp	26	2	7.7%	
	Total	1038	142	13.7%	95.9%
Overall		1164	147	12.6%	

According to Iqbal et al. (2012), 52.02% to 57.80% prevalence was calculated on the ventral side of fish and 23.12% to 27.13% above the lateral line. However, in our study, it was calculated as 0.6% and 2.1% in ventral body plane and ventral, dorsal and lateral body planes.

Our study showed the highest prevalence occurred from December to January. Besides, Iqbal et al. (2001b), Iqbal et al. (2012), and Tasawar et al. (1999) detected the highest incidence of the parasite till late winter (December to April) where the water temperature remained in the range of 13-23 ºC and low parasite incidence during the summer. Ullah et al. (2018) reported heavy infestation during summer and low during winter. The impact of water temperature variations on the *Lernaea* outbreaks has also been reported by Dalu et al. (2012) and Idris and Amba (2011). Therefore, the seasonal variations in the parasite prevalence in fish species is yet an open question, and a lot more research is required to establish it. Alam et al. (2012) proposed that monthly variations could be elucidated by fish feeding, water depth, temperature, and other physicochemical parameters. We also found that the *Lernaea* infestation reaches at peak during the cold rainy season, as also reported by Fryer (1982), Mbahinzireki (1984), M'balaka (2018), and Kabata (1985).

Previously, Berry Junior et al. (1991), Raissy et al. (2013), and Stavrescu-Bedivan et al. (2014) observed that increased water temperature could influence the intensity of *L. cyprinacea*. The optimum water temperature for its development is above 20 °C, and at a higher temperature, it can increase growth and shorten generation time (Piasecki and Avenant-Oldewage, 2008). Therefore, the 25 °C temperature reported in this study is ideal for parasite propagation, while 13 °C was recorded for survival in winter. The life cycle is temperature-dependent, whereas the prevalence and intensity decrease with decreasing temperature in winter (Mancini et al., 2006; Mancini et al., 2008). Similarly, Abbas et al. (2014), reported an increase in the parasite prevalence increasing with the rise in water temperature in the summer season. The life cycle of most species of *Lernaea* is completed in 100 days at 14 °C and 21 days at 28 °C, but the optimum water temperature falls between 23-30 °C. Fryer (1982) reported that *Lernaea* in Japan lasted for approximately four weeks at 27 °C and for five weeks at 22 °C but could prevail fora longer time during the winter season as they can thrive for five to six months. Hossain et al. (2018) observed intense infestation (72%) of *Lernaea* during January -March, with the lowest (8%) during April – June. In contrast, we observed the highest prevalence (13.6%) recorded during December -January and the lowest (3.3%) during August -September.

Some researchers like Abbas et al. (2014) and Moghadam et al. (2009) presented that small-sized fishes have shown higher parasitic infection rates. Still, others believed the infection rate increases with increasing fish body weight and length. Mirzaei (2015) cited that anchor worms readily infected the small fish. This study showed that the prevalence of *Lernaea* was more significant in younger fishes but decreased with the increase of body weight. However, in our case, the parasitic prevalence increased in fish weighing higher than 2001-4000 g, and these findings are in accordance with Tasawar et al. (2009) and Wagner et al. (2002). These results are also in line with Fryer (1982) and M'balaka (2018), who reported that small-sized (fingerling) *Balitora meridionalis* (Kottelat, 1988) was less vulnerable to *L. cyprinacea* than large-sized fishes of the same species.

Similarly, Fryer (1982) reported that chances of small-sized fish like *Haplochromis spp*. which rarely exceed 150 mm, were very slim compared to tilapias, whose size may reach up to 350 mm in length. Potentially, the larger body size offers more surface area for the parasitic invasion. In Pakistan, Kanwal et al. (2012) reported that higher-weight fishes mostly got infested than the lower-weight fish samples. Tasawar et al. (2009) specified that different weight groups with varying prevalence rates could be due to differences in fish scales orientation. Innal and Avenant-Oldewage (2012) determined a problematic correlation between the fish growth rate and infestation intensity of lernaeid parasites.

According to Innal et al. (2017), both biotic and abiotic and seasonal factors include water quality, fish feeding, size, fish reproductive biology, parasite attachment preferences, and host-parasite coevolution are involved in the incidence of lernaeid parasites. Climatic factors and environmental conditions play a considerable role in the prevalence of Lernaeid ectoparasites at different locations (Ahmed et al., 2001; Buchmann and Lindenstrom, 2002) along with the stocking density (Bashak et al., 2021). Factors like ecological and climatic conditions show a significant role in Lernaeid ectoparasite incidence in various regions and localities (Ahmed et al., 2001; Ali et al., 2014; Buchmann and Lindenstrom, 2002). Infection rate and intensity also differed between various fish species, and this phenomenon may be linked to varying fish biology (Iqbal et al., 2012).

5. Conclusion

We investigated the prevalence lernaeid parasites in public and private fish farms where Indian major carps and Chinese carps are mainly cultured. During our study, we identified 12 lernaeid species in the seven most popular cultivated fish species. The outcomes provided useful insights into the lernaeid infestation and prevalence as well as potential treatment options used by the fish farmers to control to breakout. A combination of lime and $KMnO_4$ appeared to be the most effective treatment option used by the farmers. As the diversity and prevalence of *Lernaea* species are increasing in Pakistan's freshwater fish species, the *Lernaea* infestations vary with respect to farm location, body organs and water temperatures. Therefore, the *Lernaea* species identification by molecular techniques could help to control this issue in future. Furthermore, this study provides useful insights into the most prevalent types of anchor worms in edible fish species in the Pakistan aquaculture industry. Since this study provides practical details on how to identify the potential parasites in fish and the most vulnerable parts of the fish body, this study offers a suitable option for the future researchers to follow. Furthermore, these findings can be applied at the local to regional scales.

Acknowledgements

We wish to thank all fish farmers for their cooperation to provide data and specimens for this research trial. We also wish to thank Syed Ghulam Mohayud Din Hashmi, Lecturer, Geographic Information System (GIS) Laboratory, Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pattoki, Ravi Campus, Punjab, Pakistan for help in construction of map of study area.

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