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A Preliminary Study on Prevalence of Parasites in Ornamental Fish, *Carassius auratus* (Gold Fish)

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Abstract

Ornamental fishes usually mean attractive colorful fishes of various characteristics that are kept as pets in confined space of an aquarium or a garden pool for fun and fancy. In India, about 600 fresh water and marine ornamental fishes are available, which have been identified with ornamental value. However, the success of ornamental fish culture or breeding depends on the good health status of the candidate species, failure to which cause a huge loss of socked fish. Among various diseases parasitic infection is one of the most common causes of depletion of ornamental fishes and has become a threat to fresh water ornamental fish industries throughout the world in the last decade. In this connection, the present study aimed to find out the variety of parasites present in different body parts of ornamental fishes collected at random from different local fish markets in Kolkata, West Bengal, India and also to calculate the intensity, abundance and density of different parasites. The results revealed high prevalence of parasitic infection on the most common and economically important ornamental fish, gold fish (Carassius auratus). Further results demonstrated that gold fishes were vulnerable to nine protozoan and two helminthic groups of parasites. Among them, protozoans were most susceptible to gills, skin and gut. High intensity, relative abundance and density of infection were noticed for *Cryptobia* sp. and *Trichodina* sp. Other ciliates and helminth parasites manifested moderatedegree of infection. Moreover, comparing the health and water quality it can be said that temperature and poor water quality are the limiting factor for spreading of disease to these delicate ornamental fish.

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1. Introduction

Ornamental fish or living jewels became largest hobby worldwide due to their attractive colourful shape, generally tiny size, peaceful behaviour and ability to be accommodated in confined spaces. In recent past, 20th century onwards, aquarium keeping and rearing of ornamental fish got worldwide recognition [1, 2]. However, in present days this technique had developed in to an extensive and global component of international trade worth millions of dollars. Considering the diversity of ornamental fishes, India is not much behind from the other countries. A variety of fresh water and marine ornamental fishes are available of which about 600 species had been identified as potential fishes with ornamental value. In India, the culture of ornamental fish for the aquaria is a good economic activity which has not so far been popularized yet. Since, ornamental fish trade plays an important role for socioeconomic upliftment of the farmers and also a very profitable economic activity, the trade deserves scientific study and development [3]. Nevertheless, the success of ornamental fish culture or breeding depends on the health status of the candidate species. In spite of being a lucrative business, the ornamental fish culture in India is experiencing huge loss due to invasion of parasitic organisms [4]. Parasitism is one of the most impacting problems with predominant signs of weight loss, disruption of reproduction or impotency, blindness, abnormal behavior, epithelial lesions, deformities of gills and other symptoms that result in economic loss in ornamental fish industry [5]. Since, some of the districts in West Bengal played pioneer role in aquarium fish production in the country, the business in the state is not exempted from this serious problem. Therefore, eradication parasitic infection is necessary for running this trade commercially and successfully.

Scanty informations are available from number of discrete studies around the world that protozoan, helminth and crustacean parasites cause severe diseases to the fishes [6]. Infections are evident in the skin and gill causing loss of mucus and hemorrhage at the base of the dorsal, pectoral and caudal fin [7]. A review work from European countries revealed that while, the protozoan ectoparasites like *Ichthyophthirius multifiliis, Ichthyobodo necatrix, Chilodonella cyprini, Oodinium limneticum, Trichodinids* were found to infect the ornamental fishes of European countries; external worms *Dactylogyrus extensus, Gyrodactylus bullutarudis*; and crustaceans (parasitic copepods, *Argulus japonicus*, *Argulus foliaceus*, *Lernaea cyprinacea*) were the most common external arthropod parasites of aquarium fishes [8]. A number of studies were undertaken for identification of external parasites in some ornamental fish [9-11], Eimeria spp. Cryptosporidium spp in Iran. However, *Tetrahymena corlissi*, *Thecamoeba* spp., *Giardia* spp., *Myxobolus* spp. and two metazoan parasites consisting of *Nematoda* spp. and *Benedenia monticelli* were identified as endo-parasites from a histopathological study conducted in Iranian freshwater ornamental fishes. Moreover, heavy mortality in major carp fry and fingerling due to ciliate ectoparasites like Trichodina sp., Ichthyopthirius sp., Chilodonella sp. and fluke like Dactylogyrus sp. was reported in freash water fish of Srilanka during nursery operation [12].

In India, due to parasitic infestation with *Trichodina* sp., *Myxobolus* sp, and *Dactylogyrus* sp. mortality of fish was observed from the nursery pond [13]. Another comprehensive icthyoparasotological survey in some ornamental fish farms of different parts of India, had demonstrated prevalence of three trichodinid parasites from the gills of Oranda Gold Fish (*Carassius auratus auratus* L.) of which *Trichodina* sp. were the most prevalent parasite which infects the fish during the post-monsoon season. Another recent study had revealed intensified occurrence of morphological lesions in the gill, skin, and fin of goldfish exposed to myxsporiansis leading to respiratory insufficiency and caused mass mortality in severe cases of infected fish [3]. However, to our knowledge, no detailed study was undertaken to unearth the diversity of ecto-and endo-parasites in host specific manner. Therefore, the present study aimed to the present study aimed to find out the diversity of parasites present in different body parts of ornamental fishes collected at random from different local fish markets in Kolkata, West Bengal, India and also to calculate the intensity, abundance and density of different parasites.

2. Research Method

2.1 Study location

The fresh water ornamental fish are collected at random from different local fish markets in Kolkata, West Bengal, India. Fishes from different location were selected for isolation and identification of protozoan parasites from gill, skins and tail fin. The fishes were brought alive to the laboratory for examination. All fish were kept in several aerated covered glass aquaria of 20 lit capacities, laboratory of Dept. of Zoology, Vidyasagar College, Saltlake Campus, Kolkata.

2.2 Parasitological examination

2.2.1 Isolation of pathogens

In the present study, more than 200 fishes were analysed for detection of parasitic infection. Parasitological examination was carried out for the detection and identification of the external parasites on the skin, gills and fin and gut of the samples for internal parasites.
The fish were collected throughout the year and examined in the laboratory following standard protocol. Gill, body and tail fin smear were prepared on grease free clean slides with a drop of 0.9% NaCl solution and air-dried.
The protozoan parasites were stained with Delafield Hematoxylin [Francis Delafield, 1841-1915]. The ciliophoran parasites were stained with silver impregnation technique following Klien (1958; 14).
The water quality parameters like water temperature, pH, dissolved oxygen, free carbon dioxide, alkalinity and salinity have also been measured following standard methods. For analysis of dissolved oxygen, water was collected from column region in DO bottles and fixed with $MnSO_4$ and alkaline KI. For measurement of water temperature and pH the mercury thermometer and Pen pH meter were used respectively.

2.2.3 Preparation and Staining

Fish samples were taken in live condition, killed and examined immediately for protozoan study. To reveal parasites from different sites of the body a number of techniques were undertaken.
(1) A clean spatula was held to the body of each individual and it was drawn backwards towards the tail in a smooth movement and lifting of a small amount of mucous from the different sites of the body for investigating parasites from skin. Later on for each sites, scrapped mucous was placed on a clean glass slide and examine under the 10x for parasitic investigation.
(2) In gill biopsy, a fine scissor was used to cut and open the operculum from both sides to reveal the opercula cavity. Gill filaments were taken out by cutting off the two ends of the gill arches and the whole gill filaments were dissected using fine scissor.
(3) In gut biopsy, a vertebrate scissor was used to cut the ventral side of fish and take out the gut portion and kept on petridish in vertebrate saline. Cut the gut of fish with the fine scissor and also scrapped the inner gut wall and impression smear of gut were prepared. Furthermore, three to four drops of the preparation were placed on the grease free glass slide and mixed with two drops of vertebrate saline and left to semidry.Then the slides were fixed in Schaudinn's fluid for 30 mins and preceded for staining for protozoans [15].

2.2.4 Analysis of Parasitic Infestation

The analysis of parasitic infestation for finding the incidence, intensity, density and index were carried out by following formulae [16]:

- **Incidence of Infection (W)** $= \dfrac{\text{No. of parasites} \times 100}{\text{No of Infected host}}$

- **Intensity of Infection (x)** $= \dfrac{\text{No. of parasite collected in a sample}}{\text{No of Infected host}}$

- **Density of Infection (y)** $= \dfrac{\text{No. of parasite collected in a sample}}{\text{Total host examined}}$

- **Index of Infection (z)** $= \dfrac{\text{No. of host infected} \times \text{No. of parasites collected}}{\text{Total host examined}}$

- **Relative abundance** $= \dfrac{\text{No. of each parasite} \times 100}{\text{Total no. of parasite}}$

3. Results and Analysis

In the present study, 200 gold fishes were collected from differnt markets in and around Kolkata Metropolies viz, Galif Streat Market Sonarpur Bazar (b) Sealdah Bazaar 1 (c) Sealdah Bazar 2 (d) Sealdah Bazar 3 (e). Further analysis revealed that among the five collection sites, the incidence of infection was high in site (b) and site (e). The results were represented in Figure 1.

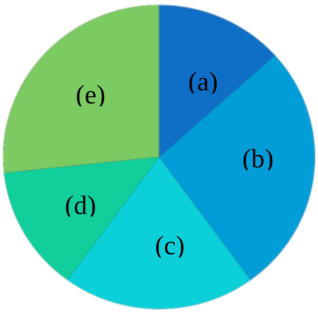


Figure 1: Incidence of infection of parasites in gold-fishes collected from different market at Kolkata

Name of parasite	No. of fish examined	No. of infected fish	No of collected parasite	Relative abundance (%)	Intensity of infection	Density of infection	Index of infection (%)
Trichodina	200	137	9060	10.52	66.13	45.3	68.5
Tetrahymena	200	137	13626	15.83	99.45	68.13	
Chilodonella	200	137	7690	8.93	56.13	38.45	
Cryptobia	200	137	16549	19.22	120.8	82.74	
Capriniana	200	137	977	1.13	7.13	4.88	
Ichthyophthirius	200	137	5708	6.63	41.66	28.54	
Piscinoodinium	200	137	10804	12.55	78.86	54.02	
Dactylogyrus	200	137	776	0.90	5.66	3.88	
Gyrodactylus	200	137	337	0.43	2.45	1.68	
Ichthyobodo	200	137	1306	1.52	9.53	6.53	
Coccidia	200	137	2018	2.34	14.73	10.09	
Microspoiadia	200	137	17243	20.02	125.87	86.21	

Table 1- The comparison between overall abundance (%), relative abundance, intencity of infection and density of infection in C*arassius auratus.*

The infected fishes were examined in the laboratory following standard protocol. Among the fises examined, 137 fishes were found tobe infected by parasitic organism representing an overall infection of nearly 68.5%. Further investigation revealed infestation of twelve parasitic species of which nine were protozoans – *Trichodina* sp., *Tetrahymena* sp., *Chilodonella* sp., *Cryptobia* sp., *Capriniana* sp., *Ichthyophthirius* sp., *Piscinoodinium* sp., *Ichthyobodo* sp., *Coccidia* sp ; two platyhelminthes – *Dactylogyrus* sp., *Gyrodactylus* sp and a single fungal species – *Microsporidia* (Table 1; Figure 2, 3) . The comperative account of relative abundance, intencity of infection as well as density of infection is represented in Table 1.

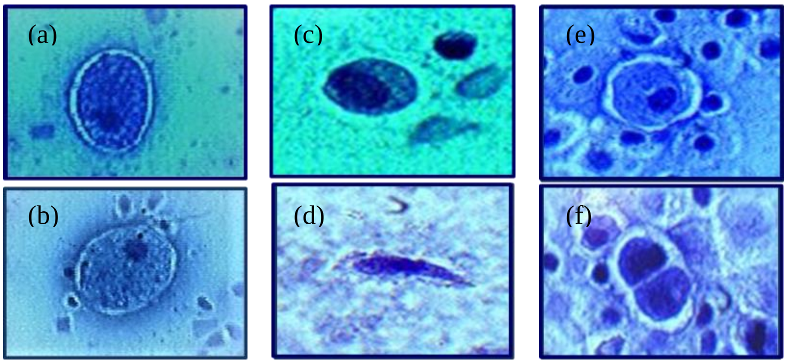


Figure 2. Photomicrogtaphs showing different protozoan parasites collected from C*arassius auratus.* a, b *Tetrahymena sp;* c,d Different developmental stages of *Cryptobia* sp; e trophozoite stage of *Ichthyophthirus* sp; f gametocyte stage of *Ichthyophthirus* sp.

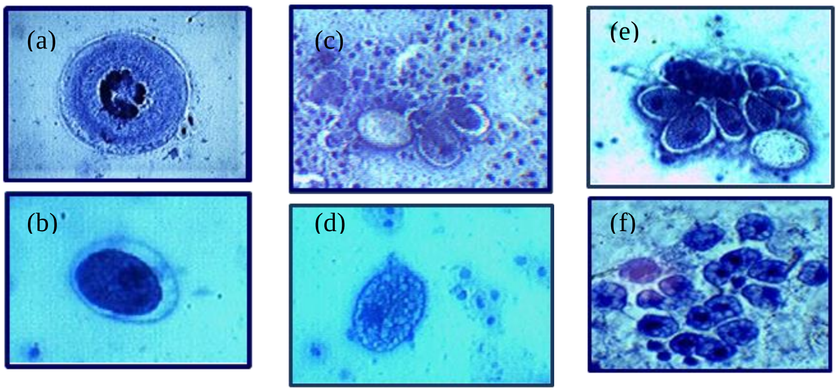


Figure 3. Photomicrogtaphs showing different protozoan parasites collected from C*arassius auratus. a Tricodina* sp; b *Piscinodium* sp; c *Ichthyobodo* sp; d coccidian parasite; e *Chilodonella* sp; f *Microsporidia.*

It is evident from the present study (Figure 4) that the relative abundance of some protozoan parasites viz. *Cryptobia* sp., *Tetrahymena* sp. and *Micrisporidia* sp. was found to be higher whereas that of *Ichthyobodo* sp., *Coccidea* and both the helminths were minimum.

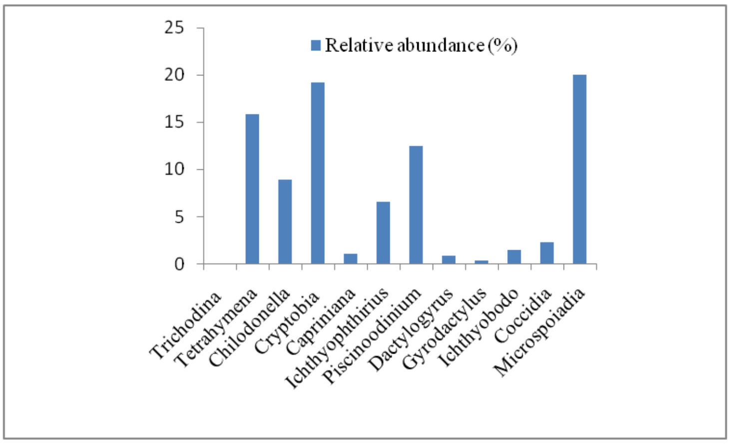


Figure 4: Relative abundance of different parasites isolated from C*arassius auratus.*Relative abundance of *Microsporidia* was maximum whereas that of the helminth parasite *Gyrodactylys* was minimum.

The analysis of the data in Table 1 revealed high density of *Cryptobia* infection in goldfish. The density of *Tetrahymena* sp, *Piscinodium* sp and Tricodina sp comes next to *Cryptobia*. However, the coccidian parasites are found to be least dense to infect the fish (Figure 5). The present study also showed that the intensity of protozoan and fungal infection in C*arassius auratus* was much higher than that of the helminth infection. However, within protozoan infection, intensity of infection was maximum for *Cryptobia* sp followed by *Tetrahymena* sp and *Picinodium* sp (Figure 6). Moreover, prevalence of the parasites on every month has been analysed and representative data were graphically plotted (Figure 7) showing incidence of presence of each of the parasite on representative slide.

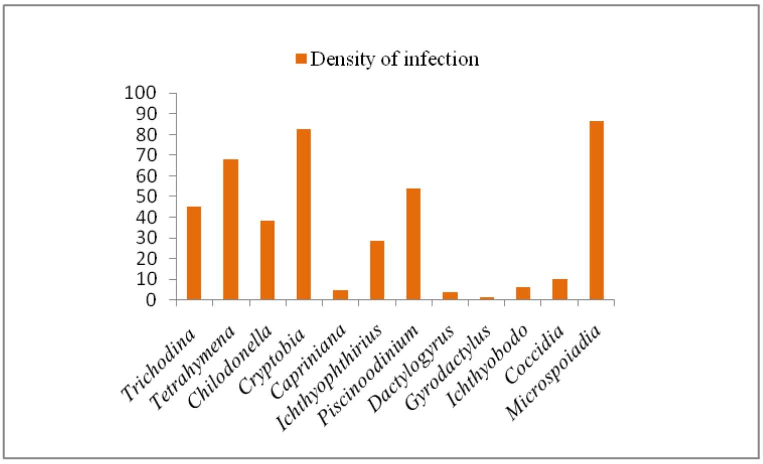
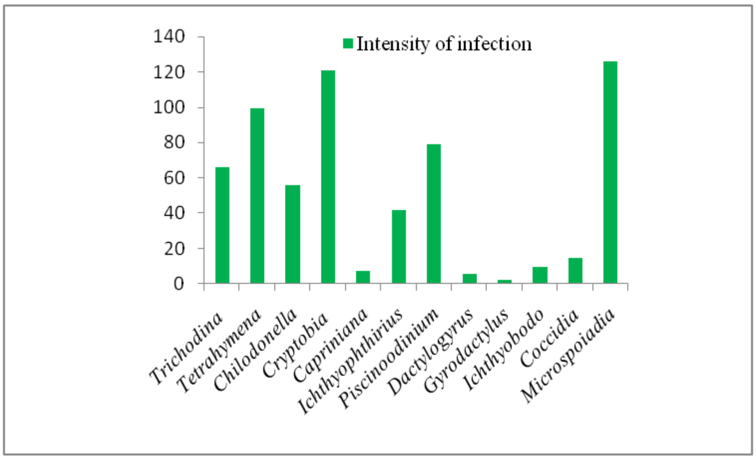


Figure 5: Density of parasitic infection in C*arassius auratus.*



Fiigure 6: Intensity of infection of different parasites of C*arassius auratus.*

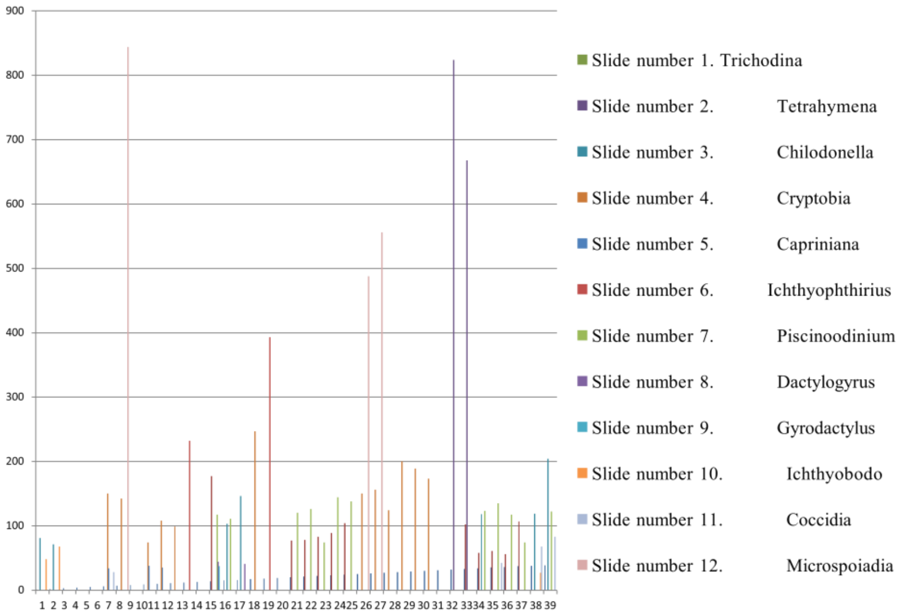


Figure 7: Graphical representation of prevalence of the infective parasites of C*arassius auratus* in each slide. Twelve slides, each representative of one month had taken and analysed for parasitic infection.

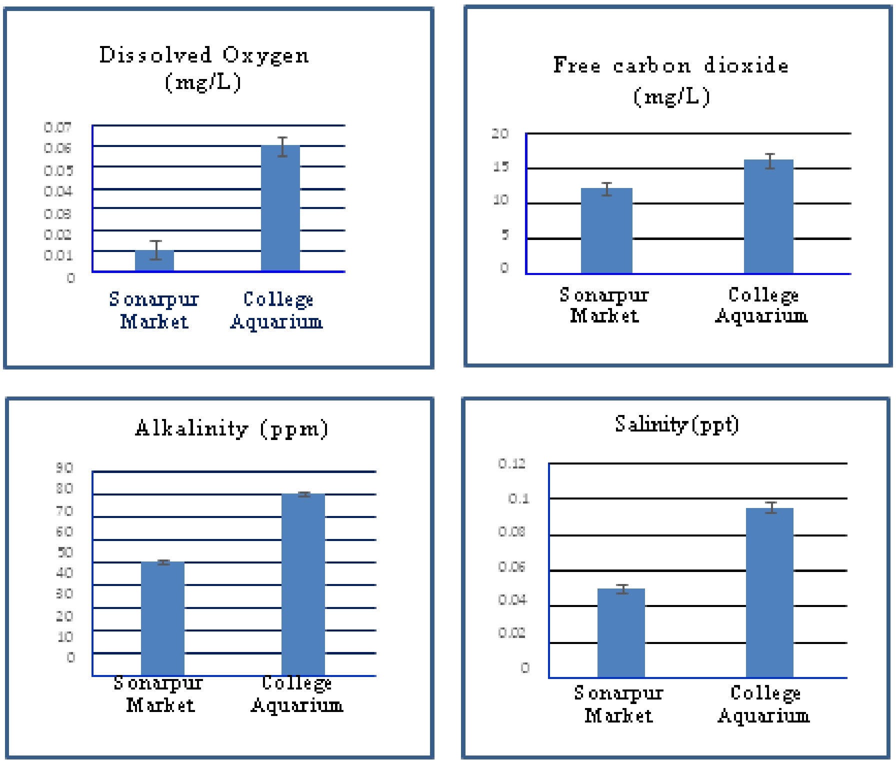


Figure 8: Graphical representation of comparison between different the water paramenters like dissolved O_2, free CO_2, alkalinity, salinity in water collected from farm (from Sonarpur market) and water maintained in laboratory condition (aquarium water).

To check the relationship between the occurane of parasitic infececton and water quality of the farm in which they are maintained, the water paramenters like dissolved O_2, free CO_2, alkalinity, salinity of the water were compared with that of the college aquarium (laboratory condition; Figure 8). Interestingly, the incidence parasitic infection was found to be significantly lower in the college aquarium (Figure 9) compared to market site, probably because of the better quality of the water maintained in the college aquarium.

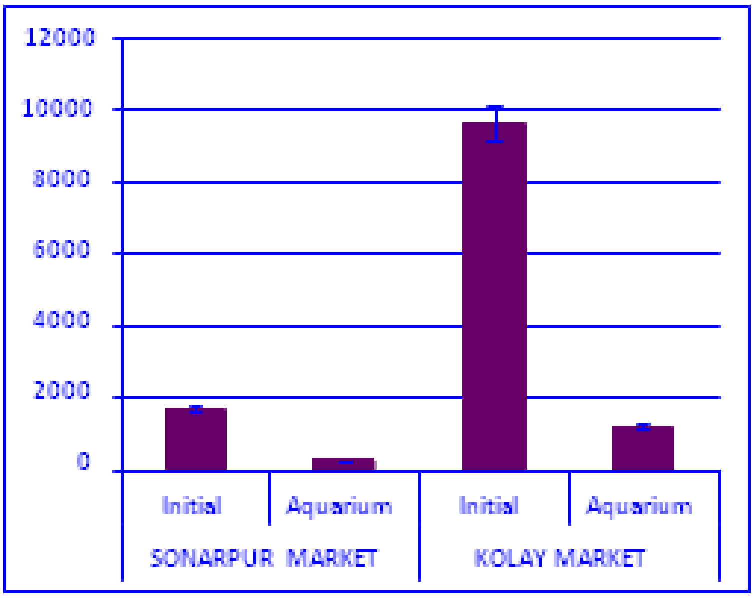


Figure 8: Graphical representation of comparison between incidence of parasitic infection in two different farm fish and the same maintained n aquarium water.

Discussion:

In the present study an attempt has been taken to study a wide spectrum of parasites infecting one of the most common and economically important ornamental fish in India, C*arassius auratus* or the gold fish. Usually the parasites cause diseases in ornamental fishes since; they are more delicate and susceptible to infection and may die very quickly.

The present findings also indicated that *Carassius auratus* were mostly infected by a number of parasitic organisms in which protozoan were most common that infect the gill, skin and gut. The rate of infection was 68.5% and the maximum intensity of infection was by the kinetoplastid protists *Cryptobia* sp followed by the ciliates *Tetrahymena* sp and the flagellate *Picinodium* sp infection was noticed. These results are in accordance with other works done in recent past in the Indian scenario [17- 19].

According to the previous reports, ecto parasitic ciliates were the most common parasites of fishes [20], which is in consistent with the results of the current study. Although the slide based analysis of the data has pointed out maximum infestation by *Cryptobia* sp, that of the ciliates like *Tetrahymena* sp and *Trichodina* sp were also considerable high. These data were in aggrement with some previous findings, where the authors have reported that *Trichodina* sp. were not only the most prevalent ciliates; but they infect the gold-fish during the post-monsoon season when the temperature is low [17, 21]. Although, our analyses showed moderate rate of infection of *Ichthiopthirus* sp throughout the year according to these authors these ciliophores infection was predominantin all seasons. However, Majumder *et al*., 2013 [22] suggested that low temperature is susceptible to this parasitic infection. Therefore, variation in the intensity of these parasites may be due to temperature fluctuation.

Previous workers have suggested significant myxozoan infection in major carp and gold-fishes in West Bengal and different parts of India [23-25]. On the contrary, in our study, myxozoan infection was not evident. This happens perhaps because myxozoan infection is well pronounced in fishes that are closely associated with common carps. Myxosporideans breaks the host specificity and transfer to the genus *Carassius* through the carp family [26], and in our case the conditionmay be different.

In the present stydy, *Gyrodactylus* sp. and *Dactylogyrus* sp. were found as monogeneans; hermaphroditic ectoparasites are having a direct life cycle and requiring only one host on which they attach to the skin and gills. These monogenean flukes are the most harmful groups affecting skin and gills of fishes and that induce irritation, destruction of gills, anorexia and impaired breathing [27]. The synergistic action of the protozoan and helminth parasites may cause large scale mortalities to these economically important ornamental fishes

[1]. Since, parasitic infections is directly co-related with the e value of the fish, special considerations are needed to be paid on this issue to get sustainable aquaculture production.

It is well accepted that due to the temperature fluctuation and water quality of post to pre-monsoon season, the fish becomes more affectedby diseases in these two seasons. High bacterial loads provide abundant food for protozoans as well as helminths parasites, which subsequently proliferate on host and cause pathology related problems. Therefore, temperature; DO; CO_2; alkalinity; salinity are the limiting factors for parasitic infection. A pilot experiment in the laboratory indicated that the water-quality maintained in aquarium helped to irradicate the initialparasitic infection present in the fishes collected from farm water (Figure 8). Therefore, it can be said that acclimatization in aquarium water maintains water quality and can lower the vulnerability of parasitic infection. These findings corroborated with those of Ahmed *et al.* (1991, 28).

4. Conclusion

Parasitological assessment in ornamental fish constitutes an important endpoint to improve the fish productivity and to keep the fish health in ornamental fish farming. In recent years, ornamental fish industry is susceptible to different protozoan, helminth and fungal infection leading to a reduced crop production in India. However, environmental fluctuations and management practices such as handling, transport, drug treatment, crowding, undernourishment, fluctuating temperature and poor water quality are acting as different limiting factors. Maintaining proper quality in aquarium water the infection can be controlled and fish production can be revived. The information emerged from this study are important when design treatment and quarantine programs for one of the most important cultured aquarium fish species, gold fish.

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