Research Article

Morphometric study and identification of external parasites in *Hypophthalmichthys molitrix* breeders in Guilan, Iran

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Abstract

The parasites that normally cause fish disease under farming condition possess a direct life cycle with no need to intermediate hosts for dissemination. The ensuing infection can therefore spread rapidly inducing more damaging lesions in the external parts of host especially the gills. In this study, in order to isolate and identify foreign parasites of phytophagous fish in Guilan province, 4 farms were selected by cluster method from silver carp breeding farms around Rasht and 16 pieces of fish in 6 stages for 6 months from April to September 2020. The fish transported live to fish health lab, underwent individual clinical observation of their skin, fins and gills with at least 10 moist extended samples prepared for each part. Isolation and morphometric identification of samples were carried out via microscopic examinations. The collected data were statistically analysed using Excel version 2010 and SPSS 20 software.

All the diagrams and table contents representing the intended parameters including frequency and the means were processed by Chi-square critical value of percentage 5. The results of the study showed a similar infestation outbreak of *L. Trichodina* on the fish fins and gills with no significant difference ($p>0.05$). *Dactyl gyrus spp* was more prevalent in fish gills and fins than on skin ($p<0.05$) but the prevalence rate of *Dactylogyrus spp, chilodonellosis*, cryptobia and *Ichthyophthirius* on the gills of phytophagous breeders were more than the rest of examined areas showing a significant difference with that of skin and gills ($p<0.05$).

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Key words: *Hypophthalmichthys molitrix*, Fish breeders, external parasites, Guilan

Introduction

Having endowed with suitable ecologic condition, Guilan province is a forerunner in warm water fish farming among the rest of provinces. As a phytophagous fish**,** silver carp

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(*Hypophthalmichthys molitrix*) belongs to *cyprinid* family (Blazhekovikj and Stojanovski, 2015; Chandra, 2004; Hussain, 2005). The fish is native to China and eastern Siberia, introduced to other parts of the world for propagation and farming. As an economically important fish in Iran, phytophagous species provide good meat quality and quantity (Aksoy and Dorcu, 2006; Jeney and Jeney, 1995; Malmberg *et al*., 2007; Muhammed, 2000). Exploitation of inland waters for fish farming purposes is one of the means in meeting protein needs. The national fisheries figures suggest that the fish production is less than 5.4 tons per hectares that is well below than that of in developed countries (Ghorbanzadeh and Nazari (2013). To develop the need to increase the density per unit area, it seems imperative therefore, to pay excessive attention to sanitary fish farm management (Karunasagar *et al*., 2003; Tekin-Özan *et al*., 2008). Safeguarding the breeder fish against pathogens in fish farms is the most important and effective means of averting damages induced by disease outbreaks (Blazhekovikj and Stojanovski, 2015; Hussain, 2005; Jeney and Jeney, 1995; Malmberg *et al*., 2007). Creating a completely pathogen –free condition in fish rearing environment is almost impossible. Nevertheless, attempts for providing a suitable condition results in decreased dangers coming from parasitic pathogens (Gussev, 1985; Jeney and Jeney, 1995; Malmberg *et al*., 2007). Due to their being kept in limited farming spaces, phytophagous breeders are prone to environmental stress and the ensuing ailments. The heightened environmental stresses cause and/ or pave the way for the weakened immune system of fishes thus giving way to diseases and secondary infection occurrences, finally resulting in fish mortalities (Malmberg *et al*., 2007; Rahanandeh, 2021; Woo, 2006). In unnatural environments, pathogens in affected fishes are, more rapidly conveyed to other fish because of high fish density and availability of potential hosts, which could exacerbate even further by the increased parasitic infestations and injuries (Jha and Bhujel, 2012; Molnar, 1994). With high infestations, parasites not only induce severe stress but also act as the primary disease causing agents inducing lesions such as hemorrhages, obsessions, hyperplasia, acute inflammations, swollen filaments and cellular secretions in brunches (Noga, 2010; Noor, 2016; Rahanandeh *et al*., 2010). These lesions might entail bacterial and/or viral diseases in fish affecting their growth rate, reproduction and physiology (Jeney and Jeney, 1995; Klinger and Francisbyd, 2005; Noga, 2010; Rahanandeh *et al*., 2010). The aim of the present study is therefore, to identify and morphometric detect the list of specific and nonspecific parasites of such fish species in fish farms to prevent parasitic infestations in farmed fish through taking suitable precautionary measures and therefore creating ideal situation for the growth and reproduction of the examined fish breeders (Fig. 2-14).

Materials and methods

The study conducted in silver carp farming ponds located around Rasht during a six months period from April –Sep 2020 in which phytophagous breeders (*Hypophthalmichthys molitrix*) underwent random selection in six

stages and each stage included 16 pieces of fish With age 3 years and weight 4-6 kg caught by fishing net. The fish samples immediately transferred live to aquatics health/disease laboratory in especial portable tank containing the same pond water, equipped with suitable aeration and air stone. In the laboratory, fish breeders were removed from the tank and then placed in a plastic tub containing water. As in the usual scientific methods of Molnar (1994), Jalali (1998) and Gussev (1985) using scissors, scalpel, lamella, glass slide and 6% physiological saline, at least 10 wet mounts samples from gill, skin and fin tissues Produced from fish breeder. In order to eliminate the excess movement of liquid particles in some samples, 1.5% agar was used. *Shaolin* solution used to fix and increase the clarity of monogeneans such as *cryptobia Brachialis* and *ammonium picrate* was used for polygene. Isolation and morphometric identification of the samples was conducted using the loop and optical microscope (Nikon with magnification rates 4, 10, 40 and100 respectively). In order to the importance of time, wet slides were prepared for microscopic examinations as quickly as possible to avoid tissues shrinkage. Ammonium picrate was used as fixative agent for samples requiring more examination time like metazoan. After wrapping the Samples with lamellas, their sides were fully dried by dryer pad. The four corners of lamellas were fixed to the slides by Entellan, followed by a drop of Ammonium Picrate put on the lateral sides of the glass slide. Due to viscosity difference of the solution with the water between lamella and the slide, the solution slowly penetrated the layer between lamella and the slide, covering the parasites on all sides after 1hr. Next, the sides of the slide were again covered with *Canada balsam stick* to keep it unchanged against any seepage for longer duration. To examine parasitic crustaceans, the parasites were first picked by forceps as they usually have a head on entry into their host's body. The tissues adhered to parasites were dissected by a scalpel to remove them completely. Next, upon rinsing in physiological saline solution to wash away all sorts of adhered organic substances, parasites were fixed and put to rest in alcohol solution (70%) for final diagnosis. To further increase transparency, the sampled parasites were submerged in *sodium hypochlorite* solution (2-3 min). They were rinsed with distilled water followed by staining (haematoxylin & eosin). The collected data put in Excel software programme 2010 and was next, analyzed by SPSS 20. Thus, all the diagrams and tables representing parameters related to frequency and the means were processed through Chi-square test at 0.5%.

Results

The results of this study showed that the percentages of *dactylogyrus spp, chilodonellosis*, cryptobia and *Ichthyophthirius* on the gills of phytophagous breeders were significantly of higher prevalence than those in the rest of the examined areas such as fins and skin ($p<0.05$) (Table.1). The results suggest that *Lernaea and Trichodina* had a uniform prevalence and distribution on fish skin, fins and gills and chi-

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square test showed an identical distribution proportion of these two parasites on all the three mentioned areas of the phytophagous breeders. ($p>0.05$) (Fig.1). Chi-square results also showed distributional proportion of *dactylogyrus spp* prevalence on gills and fins were higher than that of skin ($p<0.05$) with similar occurrence rates of *chilodonellosis* and cryptobia on skin and fins ($p>0.05$). Morphometric detection of parasites identified the list of specific and non-specific parasites of these fish (Fig.2-14).

Table 1. Statistical comparison of eternal parasites on the skin, fins and gills of phytophagous fish breeders

	Skin	Fin	Gill
Dactylogyrus hypophthalmichthys	0.00±0.00^{c}	19.75±2.25^{b}	80.25±10.11^{a}
Lernae cyprinacae	31.69±8.24^{a}	35.46±11.10^{a}	32.85±12.02^{a}
Gyrodactylus sprostonae	11.42±3.21^{b}	30.48±8.21^{a}	20.60±1.25^{a}
Ichthyophthirius multifiliis	36.17±8.25^{a}	20.92±3.25^{b}	42.91±3.28^{a}
Trichodina epizootica	34.09±5.32^{a}	30.23±6.25^{a}	35.69±6.37^{a}
Chilodonella pisicola	12.00±1.24^{b}	22.85±3.87^{b}	40.15±10.29^{a}
Cryptobia branchialis	12.18±1.26^{b}	21.05±3.26^{b}	60.52±9.24^{a}

The different letters (a, b, c) in the columns of the chart indicate a significant difference in tissue contamination with parasites.

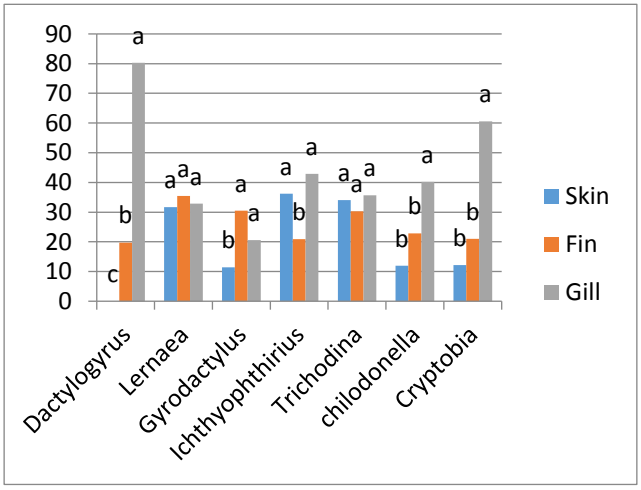

Figure 1. The prevalence percentage of external parasites on skin, gills and fins of fish breeders (The different letters (a, b, c) in the columns of the chart indicate a significant difference in tissue contamination with parasites).

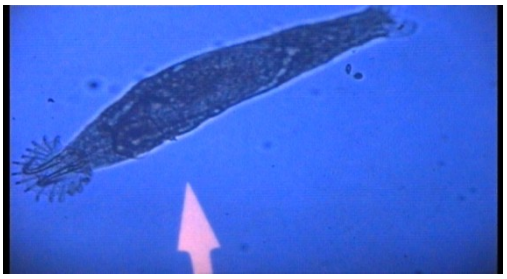

Figure 2. *Grodactylous sprostonae*

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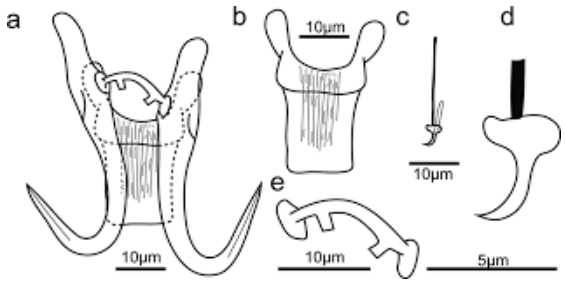

Figure 3. Morphometric characteristics *of Grodactylous sprostonae* (a) Central hook complex; (b) ventral bar; (c) hook; (d) hook sickle; (e) dorsal (Rahanandeh, 2021; Jalali, 1998).

Figure 4. *Dactylogyrus hypophthalmicht*

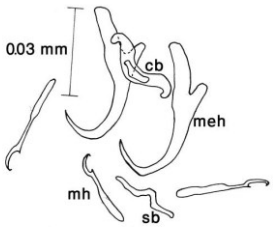

Figure 5. Morphometric characteristics *of Dactylogyrus hypophthalmichthys,* **cb**= connecting bar; **co**= copulatory organ; **meh**= median hook; **mh**= marginal hook; **sb**= supplementary bar (Rahanandeh, 2021; Jalali, 1998).

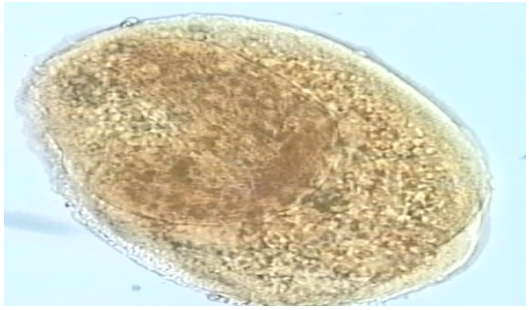

Figure 6. *Ichthyophthirius multifiliis*

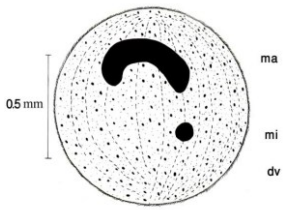

Figure 7. Morphometric characteristics *of Ichthyophthirius multifilii:* dv= digested vacuole; ma= macronucleus;mi=micronucl (Rahanandeh, 2021; Jalali, 1998).

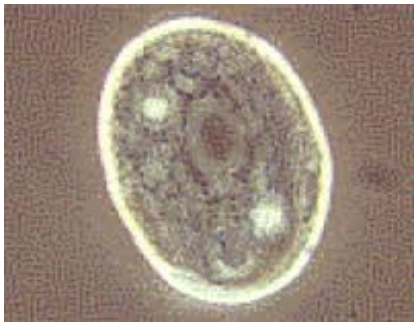

Figure 8. *Chilodonella cyprini*

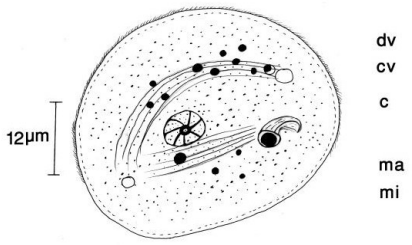

Figure 9. Morphometric characteristics *of Chilodonella cyprini* c= cytopharynx; cv= contractile; vacuole; dv= digested vacuole; ma= macronucleus; mi= micronucleus (Rahanandeh, 2021; Jalali, 1998).

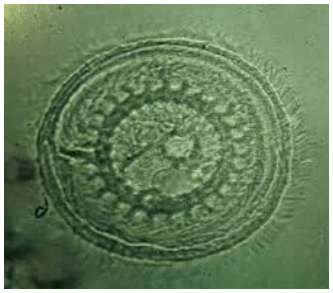

Figure 10. *Trichodina epizootica*

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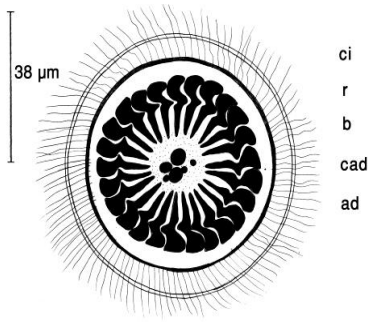

Figure 11. ad= adhesive disc; **b**= blade; **cad**= central of adhesive disc; **ci**= cilia; **r**= ray (Rahanandeh, 2021 ; Jalali, 1998).

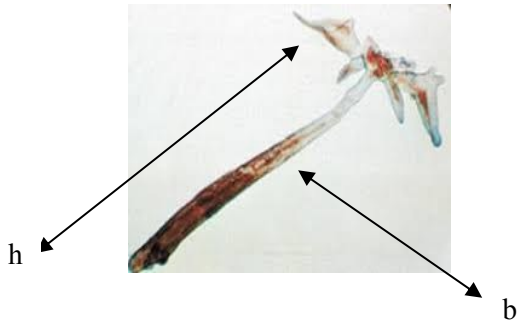

Figure 12. The front part contains the hook and the body part(b=body; h=hook) (Rahanandeh, 2021; Jalali, 1998).

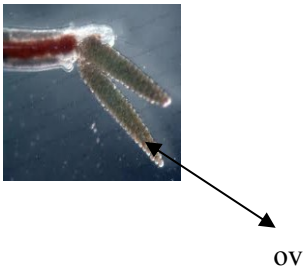

Figure 13. The posterior part contains the ovary (ov=ovary).

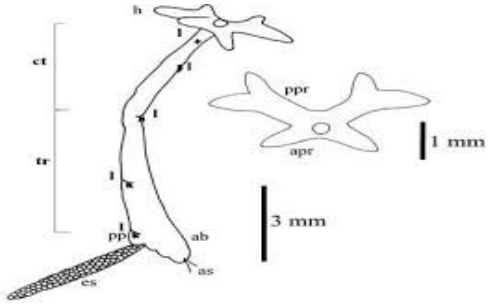

Figure 14. ab=abdomen; as= anal setae; ct= cephalothorax; es= egg sac; h= head; pp= pregenital prominence; l= legs; tr=trunk. (B) Detail of cephalic structures: apr= anterior protuberance; ppr= posterior protuberance (Rahanandeh, 2021; Jalali, 1998).

Discussion

Phytophagous propagation and culture is on the top list of fish farmers in Guilan province because of its suitable farming potentials including good quality of flesh, rapid growth rate and low feed costs. Modern data analysis in this field show that there has not been a full investigation on the vulnerability of culture ponds particularly based on physiological aspects of fish disease (Rahanandeh, 2021; Jalai, 1998). In the culture ponds of fish breeder which accommodate high population density, protecting fish against threatening pathogens is of prime importance (Jalali 1998; Molnar, 1994; Ozer and Erdem1999; Abdul-Ameer, (2004). Although parasitic infestations in fish culture farms are of less multiplicity than in natural water bodies, high culture density, limited farming space and feeding conditions within such living boundary make them liable to parasitic ailments. Karunasagar *et al*., (2003); Rahanandeh (2021); Molnar (1994) and Jalali (1998) showed that fish being reared in culture ponds are affected by various parasites. Rahanandeh (2021); Karunasagar *et al*., (2003); Abdul-Ameer (2004) and Aksoy and Dorcu (2006) showed that adverse sanitary condition , sudden changes in water temperatures and pH in farming ponds might induce parasitic

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infestations which is in conformity with the finding of the present study. Jalali and Barzegar (2006); Noor *et al*., (2007); Bhuiyan (2007); Tekin-Özan *et al*., (2008); Raissy *et al*., (2010); Rahanandeh (2021) and Nematollahi *et al*., (2013) showed that monogenean and single celled parasites can cause damages to fish fins and gills. Similar parasitic prevalence was observed in this study (Table. 1). Under unpleasant environmental condition and stress induced by parasitic infection, fish are prone to diseases. Ozer (2002) and (1999); Molnar (1994); Rahanandeh (2010) and Nematollahi *et al*., (2013) reported the presence of gyrudactylus, *dactylogyrus and lernaea* among *cyprinids*. It was observed in this study that *Gyrodactylus* prevalence on skin was %11.42, %30.48 and %20.60 on gills whereas the recorded *dactylogyrus* prevalence on skin fins and gills were %0, 19.75 and %80.25 respectively. Lernaea affecting the fish skin showed to be %31.69 followed by %35.46 and % 32.85 on fins and gills respectively (Tab.1) (Fig.1). Karunasagar *et al*., (2003) reported infestations by a variety of parasitic species in tropical fish representing similar parasite species detected in the present study. In the study, monogenean trematode of *dactylogyrus* and *Gyrodactylus* detected on the gills, skin and fins of phytophagous breeders showed significantly different prevalence rates on fish skin, fins and gills. Such severe infection could entail negative repercussions on the fish reproduction, nutrition and growth rate (*p<0.05*). Molnar (1994); Jalali and Barzegar (2006); Nematollahi *et al*., (2013) and Christoffersen *et al*., (2017) reported monogenean parasite outbreaks among carps could result in certain repr oductive complications. This study found that the prevalence rates of *Ichthyophthirius multifiliis, Chilodonella cyprini. Cryptobia branchialis and Trichodina epizootica* were meaningfully different on fish skin, fins and gills that could possibly entail sever damages to breeder fish. (*p<0.05*) (Table.1) (Fig.1). The results obtained in this study showed the prevalence percentages of single cell parasites and poly cell parasites on breeder fish might hamper biological and physiological functioning and result in their lower fecundity. Sanitary management measures including provision of safe water, suitable feeding, and hygienic disinfection procedure of fishponds and preventing the entry of wild fish or any other disease vectors in culture ponds might drastically reduce parasitic infestations in breeder fishponds.

This study, for the first time, using morphometric detection of parasites, identified a list of specific and non-specific parasites of this species of fish breeders (Fig. 2-14).

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Conflicts of interest

None of the authors has any conflicts of interest to declare.

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