

## Research Article

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

M. Rahanandeh<sup>1\*</sup>, B. Tizkar<sup>1</sup>

<sup>1</sup>Guilan Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran

Received: May 2021

Accepted: July 2021

## 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 ( $p<0.05$ ).

**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

\*Corresponding author's Email:  
rahanandeh1340@gmail.com

(*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 (Gusse, 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 bristles (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 and 100 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-

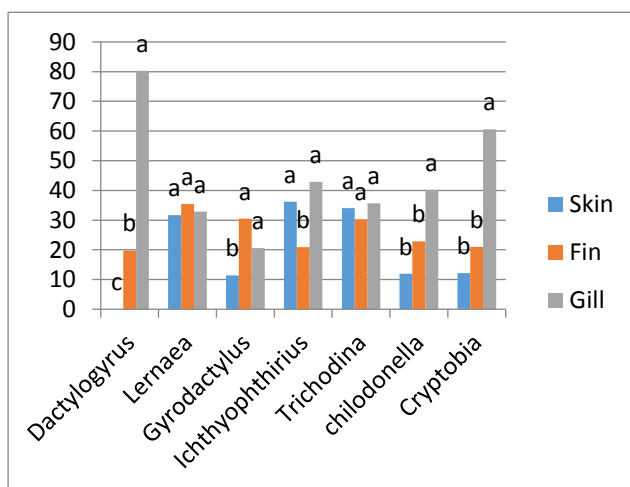
square test showed an identical distribution proportion of these two parasites on all the three mentioned areas of the phytophagous breeders. ( $p>0.05$ ) (Fig.1). Chi-square results also showed distributional proportion of *dactylogyrus spp* prevalence on gills and fins

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).

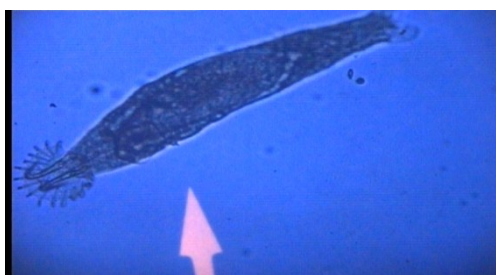
**Table 1.** Statistical comparison of eternal parasites on the skin, fins and gills of phytophagous fish breeders

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

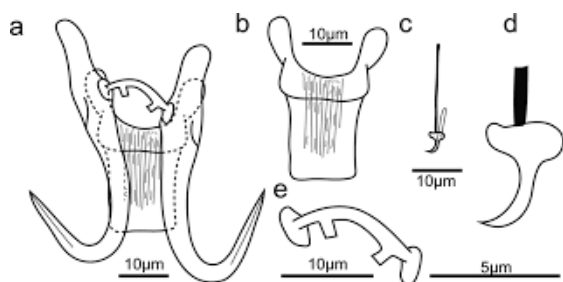
The different letters (a, b, c) in the columns of the chart indicate a significant difference in tissue contamination with parasites.



**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).



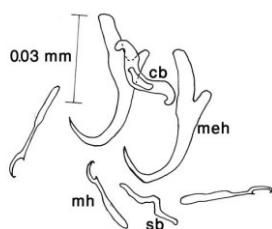
**Figure 2.** *Grodactylous sprostonae*



**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).



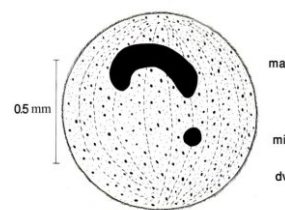
**Figure 4.** *Dactylogyrus hypophthalmicht*



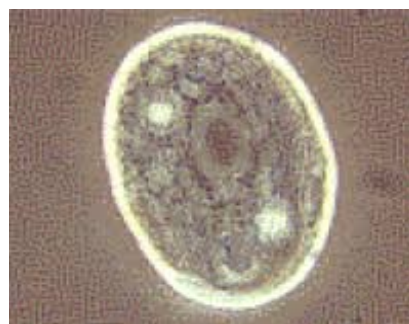
**Figure 5.** Morphometric characteristics of *Dactylogyrus hypophthalmichthys*, **cb**= connecting bar; **co**= copulatory organ; **meh**= median hook; **mh**= marginal hook; **sb**= supplementary bar (Rahanandeh, 2021; Jalali, 1998).



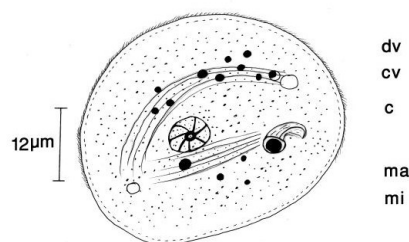
**Figure 6.** *Ichthyophthirius multifiliis*



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



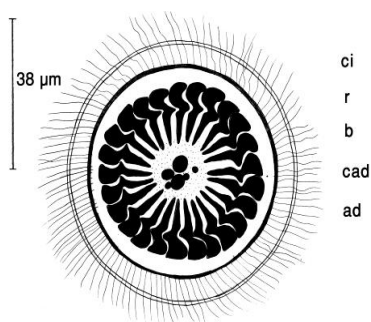
**Figure 8.** *Chilodonella cyprini*



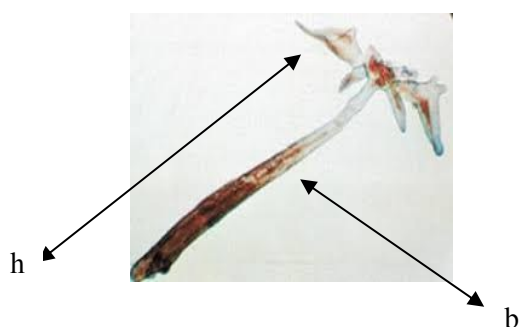
**Figure 9.** Morphometric characteristics of *Chilodonella cyprini* c= cytopharynx; cv= contractile; vacuole; dv= digested vacuole; ma= macronucleus; mi= micronucleus (Rahanandeh, 2021; Jalali, 1998).



**Figure 10.** *Trichodina epizootica*



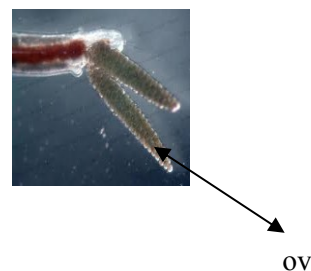
**Figure 11.** ad= adhesive disc; b= blade; cad= central of adhesive disc; ci= cilia; r= ray (Rahanandeh, 2021 ; Jalali, 1998).



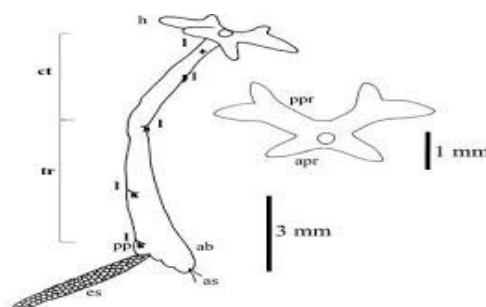
**Figure 12.** The front part contains the hook and the body part (b=body; h=hook) (Rahanandeh, 2021; Jalali, 1998).

## 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 Erdem 1999; Abdul-Ameer, (2004).



**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).

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

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 *Gyrodactylus*, *dactylogyrus* and *lerna* 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 reproductive 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 severe 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).

### Acknowledgment

We are very grateful to the management of silver carp, Mr. Khoshhal, for helping us with this study.

### Conflicts of interest

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

### References

Abdul-Ameer, K.N., 2004. The first record of the ciliated protozoan *Trichodina cottidarum* in Iraq on the gills of the common carp (*Cyprinus*

*carpio*). *Ibn Al-Haitham Journal For Pure And Applied Science*, 17(3): 1– 6.

Aksoy, S., Saglam, N. and Dorucu, M., 2006. External parasites of three cyprinid fish species from Lake Hazar in Turkey. *Indian veterinary journal*, 83(1), 100-101.

Bhuiyan, A.S., Akther, S. and Musa, G.M., 2007. Occurrence of parasites in *Labeo rohita* (Hamilton) from Rajshahi. *University journal of Zoology, Rajshahi University*, 26, 31-34. DOI:10.3329/ujzru.v26i0.694

Blazhekovikj, D.D. and Stojanovski, S., 2015. Silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) as hosts of *Sinergasilus polycolpus*, a new copepod species in the ichthyoparasitofauna of Macedonia. *Food Science and Quality Management*, 45, 32-38.

Chandra, K.J., 2004. Fish Parasitology. Lima Printing Press, Mymensingh, p: 176.

Christoffersen, T.B., Kania, P.W., von Gersdorff Jørgensen, L. and Buchmann, K., 2017. Zebrafish *Danio rerio* as a model to study the immune response against infection with *Ichthyophthirius multifiliis*. *Journal of fish diseases*, 40(6), 847-852. DOI:10.1111/jfd.12543

Ghorbanzadeh, R. and Nazari, S., (2013) Ian Fisheries Organization, Statistical Yearbook, pp. 28. (In Persian)

Gushev, A.V., 1985. Parasitic metazoans: Class Monogenea. In: Bauer, O.N. (Ed.) Key to the parasites of freshwater fish fauna of the U.S.S.R. Nauka, Leningrad, 2, 1-424.

Hussain, H.T., 2005. Ectoparasitic infection of the common carp and silver carp Fingerlings stocked during winter in AL-Shark AL-Awsat fish farm, Babylon Province, Iraq. M.Sc. thesis. Coll. AL-Mussab Technical Foundation of Technical Education: pp.106.

Jalali, B. and Barzegar, M., 2006. M. Fish parasites in Zarivar Lake. *Journal of Agricultural Science and Technology*, 8, 47-58.

Jalali, B., 1998. Parasites and parasitic diseases of Iranian freshwater fish, Iranian Department of Aquaculture, Iran Fisheries Publications, Tehran, Iran 1 Ed.p:155-167. (In Persian)

Jeney, Z. and Jeney, G., 1995. Recent achievements in studies on diseases of common carp (*Cyprinus carpio* L.). *Aquaculture*, 129: P: 397–420. DOI:10.1016/0044-8486(94)00283-T

Jha, D.K. and Bhujel, R.C., 2012. Incidence of fish diseases and management practices in Nepal. *Small-scale Aquaculture for Rural Livelihoods*, 160-165.

Karunasagar, I., Karunasagar, I. and Otta, S.K., 2003. Disease problems affecting fish in tropical environments. *Journal of Applied Aquaculture*, 13(3-4), 231-249. DOI:10.1300/J028v13n03\_03

Klinger, R. and Francisbyd, R., 2005. Introduction to fresh water fish parasites. University of Florida, P: 162-163.

Malmberg, G., Collins, C., Cunnigham, C. and Jalali, B., 2007. *Gyrodactylus derjavinoidea* sp.nov. (Monogenea, Platyhelminthes) on *salmo*



*trutta trutta* L. and G. Derjavini Mikailov, 1975 on s.t. caspius Kessler, two different species of *Gyrodactylus*- Combined morphological and molecular investigations. *Acta parasitology*, 52(2), 89-103. DOI:10.2478/s11686-007-0016-1

Molnar, K., 1994. Effect of decreased water oxygen content on common carp fry withm D. vastator (monogenea) infection of varing severity. *Diseases of Aquatic Organism*, 20, 153-157. DOI:10.3354/dao020153

Muhammed, S.K., 2000. An external and eye parasite survey for carp fishes in ALEskandaryia Region (Babylon). Ph.D. Thesis Coll. Vet: Med., Univ. Baghdad, P, 82.

Nematollahi, A., Ahmadi, A., Mohammadpour, H. and Ebrahimi, M., 2013. External parasite infection of common carp (*Cyprinus carpio*) and big head (*Hypophthalmichthys nobilis*) in fish farms of Mashhad, northeast of Iran. *Journal of parasitic diseases*. 37(1), 131-133. DOI:10.1007/s12639-012-0146-8

Noga, E.J., 2010. Fish disease:diagnosis and treatment, Two.Edi. Iowa State University Press, USA, 2 Ed. P: 95-97. DOI:10.1002/9781118786758

Noor El Deen, A.E., Abd El-Hady, O.K., Lila A., Mohamed and Mona, S. Zak. 2016. Trials of Control of Some External Parasitic Nile tilapia Diseases with Emphasis on Preparation of vaccine against *Ichthyophthirius multifiliis* , *International Journal of Pharm Tech Research*, 1.9(9), 130-137.

Ozer, A. and Erdem, O., 1999. The relationships between occurrence of ectoparasites, temperature and culture conditions: A comparison of farmed and wild common Carp (*Cyprinus carpio* L., 1758) in the Sinop region of northern Turkey. *Journal of Natural History*. 33(4), 483-491. DOI:10.1080/002229399300209

Ozer, A. 2002. Co-existence of *Dactylogyrus anchoratus* Dujardin (1845) and *D. extensus* (Mueller and Van Cleave, 1932) (Monogenea), parasites of common Carp (*Cyprinus Carpio*). *Helminthologia*, 39(1), 45-50.

Rahanandeh, M. 2021. A practical guide for fish health and diseases, 1Ed. P, 233-223, 1. (In Persian)

Rahanandeh, M., Sharifpour, I., Jalali, B., Kazemi, R., Aminian, B. and Shafiei, S. 2010. Survey on Dactylogyrus in Caspian Frisian roach (*Rutilus frisii kutum*) caused by *Dactylogyrus frisii*. *Global veterinaria*, 4, 515-518.

Raissy, M., Ansari, M. Lashkari A., and Jalali, B. 2010. Occurrence of parasites in selected fish species in Gandoman Lagoon. *Iranian Journal of Fisheries Sciences*, 9 (3), 464-471.

Tekin-Özan, S., Kir, İ. and Barlas, M., 2008. Helminth parasites of common carp (*Cyprinus carpio* L., 1758) in Beyşehir Lake and population dynamics related to month and host size. *Turkish Journal of Fisheries and Aquatic Sciences*, 8(2), 201-205. DOI:10.1007/s10661-007-9765-4

Woo, P.T.K., 2006. Fish Diseoses and Disorders, Volumel, Protozoan and Metozoam Parasites, CABinternational, Canada. 2Ed. P: 116- 124.