



Ecological studies and histopathological alterations caused by *Argulus japonicus* among goldfish in Mosul, Iraq

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ABSTRACT

Argulus japonicus (fish lice) is one of the the is one of the most economically significant *ectoparasites* infesting cultured and wild fish, predisposing hosts to secondary infections. This study aimed to investigated the ecological, epidemiological, and histopathological alterations caused by *A. japonicus* infestation in goldfish (*Carassius auratus*) in Mosul, Iraq. A total of 320 goldfish were obtained from fish local markets and carefully examined for infestation. Parasites were collected manually from the gills, fins, operculum, and skin using fine forceps, identified morphologically with standard entomological keys, and examined histologically using routine pathological procedures. Of the goldfish examined (320), 197 (61.56%; 95% CI: 56.13–66.72) were infested, yielding a total of 2,509 parasites. Infestation level ranged from 1 to 25, with a mean intensity of 12.73 and abundance values of 7.84. Histopathological examination revealed multiple lesions in the skin and gills of infested goldfish, including goblet cell hyperplasia, dermal edema, necrosis, hemorrhage, and gill lamellae destruction.

Keywords

Carassius auratus; Fish lice; Epidemiology; Pathological alterations

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Abbreviations

H&E: Hematoxylin and Eosin

CI: Confidence Interval

χ^2 : Chi-Square value

Introduction

Parasitic infection and infestation are recognized as one of the most significant challenges in aquaculture [1], often resulting in reduced fish growth, impaired vitality, and even death [2]. Among aquatic parasites, ectoparasites are of particular concern for aquatic animals raised in both aquaria and ponds. The major groups of ectoparasites include crustaceans, monogenes, and protozoans [3]. Of these *Argulus* species, a crustacean is a major ectoparasite with great economic importance in aquaculture [4].

Argulus species belong to the family Argulidae, a group of branchiuran parasites that infest and cause disease in fish and are commonly known as fish lice [5]. More than 100 species have been described, including *A. coregoni*, *A. foliaceus*, *A. inducus*, *A. japonicus*, and *A. siamenses* [6-8]. These parasites are known to infest a wide range of fish and amphibians, with particular affinity for goldfish, koi, centrarchids (sunfishes), cyprinids (minnows and carps), and salmonids (trout and salmon) [2-4].

Among them, *A. japonicus* causes the greatest economic impact among all ectoparasites that infest cultured and wild fishes. Infestation not only causes direct tissue damage but also causes susceptibility of its host to other infections such as bacterial (e.g., *Aeromonas* spp, *Pseudomonas* spp), parasitic (e.g., *Costia* spp), viral (e.g., *Rhabdovirus carpio*), and fungal (e.g., *Saprolegnia* spp) infections. Furthermore, *A. japonicus* can also play the role of being the intermediate host for numerous species of roundworms (nematodes) in fish populations [8, 9]. The economic losses resulting from *A. japonicus* infestation in fish are not only incurred due to the fish mortality but also from the costs of treatment and reduced growth rate during and after the outbreak of the disease, which affect the growth of fish farming worldwide [7, 9].

Ornamental fish such as goldfish (*Carassius auratus*) are highly valued for home decoration and entertainment due to their diversity and picturesque colors. The global ornamental fish industry has expanded rapidly, creating employment opportunities for many people all over the world [5, 6].

Given the importance of goldfish in the ornamental trade and the significant economic and pathological burden of *A. japonicus*, the present study aimed to investigate the ecological, epidemiological, and histopathological alterations caused by *A. japonicus* (fish louse) infestation in goldfish (*C. auratus*) in Mosul, Iraq.

Result

Out of the 320 goldfish examined, 197 (61.56%; 95% CI = 56.13–66.72) were infested with *A. japonicus*. A total of 2,509 *A. japonicus* were collected from the 197 infested goldfish. The infestation intensity ranged from 1 to 25, with a mean intensity of 12.73 and abundance values of 7.84 (Table 1). A statistically significant difference in infestation level of *A. japoni-*

Table 1. Ecological parameters of *A. japonicus* infestation among goldfish (*C. auratus*) in Mosul City, Iraq

Ecological indices	Occurrence
Number of fish examined	320
Number of fish infested	197
Total number of <i>A. japonicus</i> collected	2,509
Range of infestation	1-25
Prevalence (%)	61.56
Mean intensity	12.73
Abundance	7.84

cus among goldfish was observed ($\chi^2=34.88$; p-value < 0.01). The distribution of *A. japonicus* prevalence and intensity among goldfish followed a normal distribution curve. The highest infestation rate was recorded in the 5 to <10 parasite category, with 59 goldfish (18.44%; 95% CI = 14.57–23.05). The lowest was in the < 5 parasite category, with 14 goldfish (4.38%; 95% CI = 2.62–7.21). The number of goldfish infested with *A. japonicus* in the other infestation level categories, ranged from 31 fish with 20 to 25 *A. japonicus* (9.69%; 95% CI = 6.91–13.42) to 48 fish with 10 to < 15 *A. japonicus* (15.00%; 95% CI = 11.50–19.33) (Table 2).

Histopathological examination of goldfish skin infested with *A. japonicus* revealed severe lesions, including goblet cell hyperplasia, scale pocket forma-

Table 2. Level of *A. japonicus* infestation among goldfish (*C. auratus*) in Mosul City, Iraq

Level of infestation	Number of fish	Prevalence (%)	95 % CI	χ^2	p-value
<5	14	4.38	2.62 – 7.21		
5 to <10	59	18.44	14.57 – 23.05		
10 to <15	48	15.00	11.50 – 19.33	34.88 ¥	<0.01
15 to <20	45	14.06	10.68 – 18.30		
20 to 25	31	9.69	6.91 – 13.42		

tion and dermal edema, Zenker's necrosis of the muscular layer, myositis, hemorrhage, and actinic arrows of scales embedded within the dermis (Fig. 1).

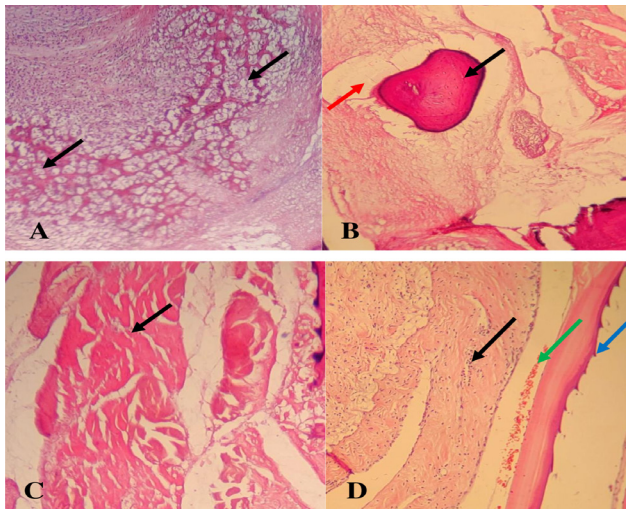


Figure 1. Histopathological examination of goldfish (*C. auratus*) skin infested with *A. japonicus*; 1a, showing hyperplasia of goblet cells (black arrows). 1b, showing scale (black arrow) and edema (red arrow). 1c, showing Zenker necrosis of the muscular layer (black arrow). 1d, shows myositis (black arrow), hemorrhage (green arrow), and actinic arrows of scales embedded in the dermis (blue arrow) H&E X40.

The histopathological lesion observed in the gills was hemorrhage and necrosis of the gill filaments (Fig. 2).

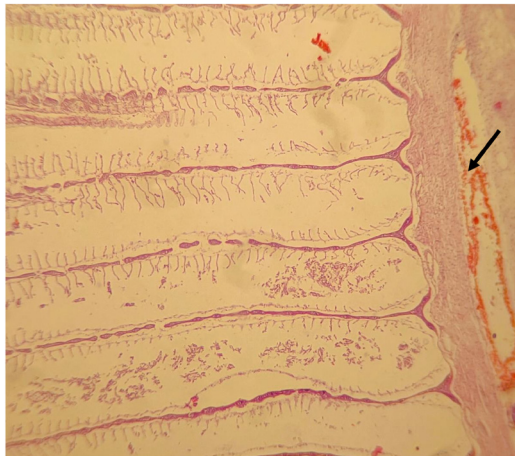


Figure 2. Histopathological examination of goldfish (*C. auratus*) gills infested with *A. japonicus*, showing hemorrhage (black arrow) and necrosis of gill filaments, H&E X40.

Discussion

In this study, we aimed at investigating the ecological, epidemiological, and histopathological alterations caused by *A. japonicus* infestation in goldfish in Mosul, Iraq. Prevalence is a valuable

and informative measure for lice epidemiology and population dynamics in fish [10]. In the present study, *A. japonicus* infestation was recorded in 61.56% of examined, a prevalence higher than 35.20%, 51.18%, and 57.00% reported among goldfish in China [9], India [6], and Indonesia [11], respectively. *Argulus japonicus* has been documented to infest a wide range of fishes, with varying prevalences of 100.00% in pike-perch (*Sander lucioperca*) [12], 80.00% in common carp (*Cyprinus carpio*) [13], and 65.00% in Koi (*Cyprinus rubrofuscus*) [11]. In addition, prevalences of 42.12% in mandarin fish (*Siniperca chuatsi*), 36.07% in black carp (*Mylopharyngodon piceus*), 35.12% in silver carp (*Hypophthalmichthys molitrix*), 33.83% in rainbow trout (*Oncorhynchus mykiss*), 31.60% in perch (*Perca fluviatilis*), 20.00% in brown trout (*Salmo trutta*) [9], and 9.50% on *Cirrhinus mrigala* [4] have also been reported.

The high mean intensity (12.73) and abundance (7.84) observed in this study. Aalberg et al. [12] and Kismiyati et al. [11] also documented a high mean intensity and abundance in Indonesia and Slovakia, respectively. Such elevated mean intensity and abundance of *A. japonicus* may be attributed to the change in the natural abiotic and biotic factors in the aquatic system as recorded in cultured fishes, which strongly influence the outbreak of ectoparasites (including lice) in fishes [14].

The high prevalence and heavy infestation rate of *A. japonicus* in this study support its classification as a generalist parasite, as postulated by many authors who reported a high prevalence and heavy infestation rate of the fish louse [9].

Histopathological analysis revealed severe alterations in the musculoskeletal system of infested goldfish, which are characterized by goblet cell hyperplasia, scale pocket formation and dermal edema, Zenker's necrosis of the muscular fibers, myositis, hemorrhage, and actinic arrows of scales embedded within the dermis. Histological findings such as infiltration of inflammatory cells and Zenker necrosis of the myofibril with hemorrhage and edema were reported in the musculoskeletal system of goldfish (*C. auratus*) infested

with *A. japonicus* [15], also Ahamad et al. [16] and Al-Darwesh et al. [15] reported similar histological findings in *Labeo rohita* and *Cirrhinus mrigala* fishes, respectively.

Infestation of also led to the loss of the anatomical structure of goldfish. The histopathological finding revealed a total destruction of the secondary gill lamellae with disintegration of the gill filament, which was shown by the intensity of the louse (*A. japonicus*) infestation. Long-standing infestation with a large number of *A. japonicus* may negatively affect the structure of the gill in goldfish. The rakers of the gill were found blocked with too many debris particles, which could have resulted in the complete loss of the secondary gill lamellae. These findings are similar to the reports of Mamun et al. [17]. The pathological effects caused by argulosis are associated with the constant irritative behavior of infected fish, erratic swimming patterns and feeding disruption, caused by lice attachment [18].

The pre-oral stylet and modified mouthparts penetration of the host's skin, muscles, and gills causing localized hemorrhage and erythema, and numerous numbers of *Argulus* species feeding close to each other may cause localized swelling and edema. Feeding activity and constant piercing of lice with their stylet (the mouthpart adopted for sucking blood), introduces cytotoxic enzymes leading to inflammatory lesions that are characterized by hemorrhages, increased mucous secretion, and necrosis of the affected area [19, 20, 22]. The gill damage causes osmoregulation disturbances and reduces the ability of the fish to sustain normal oxygen uptake, which may lead to hypoxia and even the death of the infested fish [21].

In conclusion, our findings demonstrate that *A. japonicus* is highly prevalent in goldfish (*C. auratus*) populations in Mosul, Iraq, with heavy infestation intensities. The parasite induces profound histological lesions in both the skin and gills of the infested goldfish, underscoring its significant impact on host health and its potential to cause mortality in cultured fish.

Materials and Methods

Study area

This study was conducted in Mosul, Iraq, a city located on the west bank of the Tigris River, directly opposite the ancient Assyrian city of Nineveh on the east bank [23]. Mosul lies at 43.09 E and 36.19 N, with an elevation of 230 meters above sea level, and it covers a total land area of 70 sq mi (180 km²). The city has a climate that is characterized by cold winters with occasional snowfall and dry, hot summers [24].

Goldfish collection

A total of 320 goldfish were obtained from local fish markets and fish stores in Mosul. The fish were kept alive in water vats and transported to the laboratory of the Pathology and Poultry Diseases Department, College of Veterinary Medicine, University of Mosul, Mosul, Iraq, for parasitological, pathological and histopathological examinations.

Collection and identification of *Argulus japonicus*

Argulus japonicus was collected manually from the gills, fins, operculum, and skin of each goldfish using fine forceps following a thorough and careful external examination. *Argulus japonicus* from each goldfish was collected and preserved separately into sample bottles containing 70% ethanol for morphological identification, their count and intensity of their infestation also been record. Identification was performed under a stereomicroscope, using entomological keys described by Bykhovskaya-Pavlovskaya et al. [25] and Rushton-Mellor [26].

Histopathological analysis

Few of the goldfish infested with *A. japonicus* were anesthetized following the method described by AL-Taee et al. [27]. Tissue samples, including muscle, skin, and gill were excised and fixed in neutral buffered formalin (10%) for a at least 48 hours. Fixed tissues were trimmed into small parts of about 2 to 3 mm sections, dehydrated through ascending grades of ethanol (70%, 80%, 90%, and 100%; 15 minutes each), cleared in absolute xylene (100%) and embedding in paraffin wax. Sections of 5 µm were prepared and stained with H&E according to the procedure of Akanbi et al. [28]. Slides were examined under a compound light microscope at X40 magnification.

Statistical evaluation

The prevalence, abundance, and mean intensity of *A. japonicus* infestation in goldfish were calculated following the ecological indices (in terms of comparative parameters) for parasitology described by Margolis et al. [29] and Bush et al. [30].

$$\text{Prevalence (\%)} = \frac{\text{Number of infected fish} \times 100}{\text{Total number of fish examined}}$$

$$\text{Mean Intensity} = \frac{\text{Number of collected parasites}}{\text{Number of infected fish}}$$

Authors' Contributions

S.K.A., D.A.A., and N.S.A. conceived and planned the experiments. S.K.A. and N.S.A. carried out the experiments. S.K.A., D.A.A., and N.S.A. contributed to sample preparation. S.K.A., D.A.A., N.S.A., SDO, and FIG contributed to the interpretation of the results.

SDO took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Competing Interests

The authors hereby state that there are no conflicts of interest among them concerning the funding of this research and the publication of this manuscript.

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