Research Article

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

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Abstract

Argulus japonicus (fish lice) has the greatest economic impact among all ectoparasites infesting cultured and wild fish, and it increases the susceptibility of its host to pathogens. This study aimed to determine the ecology, epidemiology, and histopathological alterations caused by *A. japonicus* on goldfish (*Carassius auratus*) in Mosul, Iraq. Three hundred and twenty goldfish were purchased from fish markets and fish stores in Mosul, Iraq. The fish was carefully examined, and *A. japonicus* were collected manually from the gills, fins, operculum, and entire skin of the fish with the aid of fine forceps. The collected *A. japonicus* were identified using appropriate entomological keys, and standard pathological procedures were conducted on *A. japonicus*-infested fish. Of the total number of goldfish examined (320), 197 were infested with *A. japonicus*, representing 61.56% (95% CI = 56.13–66.72). A total of 2,509 *A. japonicus* lice were collected from the 197 infested goldfish. The level of infestation ranged between 1 and 25. The mean intensity and abundance values were 12.73 and 7.84, respectively. Different pathological lesions were recorded in the skin and gills of infested goldfish. The infestation of this fish louse caused diverse histological lesions on the skin and gills of the infested goldfish.

Keywords: *Carassius auratus*; Fish lice; Epidemiology; Pathological alterations

Abbreviations

H&E: Hematoxylin and Eosin

CI: Confidence Interval

χ^2: Chi-Square value

Introduction

Parasitic infection and infestation have been ascribed as one of the most important causes of setbacks in aquaculture [1]. Disease conditions of this origin can lead to a decreased growth rate, reduced vital activity of the fish, and even death [2]. Ectoparasites are the major parasites of aquatic animals raised in both aquaria and ponds. The major groups of these parasites comprise crustaceans, monogenes, and protozoans [3]. Of these *Argulus* species, a crustacean is a major ectoparasite with great economic importance in aquaculture [4].

Argulus species of the family Argulidae are members of a large group of branchiuran parasites that infest and cause disease in fish and are commonly known as fish lice [5]. *Argulus coregoni*, *A. foliaceus*, *A. inducus*, *A. japonicus*, and *A. siamenses* are among the over 100 species of the genus that infest fishes [6-8]. *Argulus* species are known to infest a wide range of fishes and amphibians, especially goldfish, koi, centrarchids (sunfishes), other cyprinids (minnows and carps), and salmonids (trout and salmon) [2-4].

Argulus japonicus causes the greatest economic impact among all ectoparasites that infest cultured and wild fishes, and it causes the susceptibility of its host to other infections such as bacterial (*Aeromonas* species, *Pseudomonas* species, etc.), parasitic (*Costia* species), viral (*Rhabdovirus carpio*), and fungal (*Saprolegnia* species) infections. *Argulus japonicus* can also play the role of being the intermediate host for numerous species of roundworms (nematodes) in fishes [8, 9]. The economic losses resulting from *A. japonicus* infestation in fishes are not only incurred due to the death of the fish but also from the cost of treatment and decreased 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 believed to be one of the vital means of home decoration and entertainment due to their diversity and picturesque colors. Also, ornamental fish are raised for commercial reasons, and their industry is growing rapidly, creating employment opportunities for many people all over the world [5, 6].

This study aimed to determine the ecology, epidemiology, and histopathological alterations caused by *A. japonicus* (fish louse) among goldfish (*C. auratus*) in Mosul, one of the major cities in Iraq.

Results

Of the total number of goldfish examined (320), 197 were infested with *A. japonicus*, representing 61.56% (95% CI = 56.13–66.72). In total, 2,509 *A. japonicus* were collected from the 197 infested goldfish. The level of infestation ranged between 1 and 25. The mean intensity and abundance values were 12.73 and 7.84, respectively (Table 1).

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 *A. japonicus* collected	2,509
Range of infestation	1-25
Prevalence (%)	61.56
Mean intensity	12.73
Abundance	7.84

There was a statistically significant difference in the level of *A. japonicus* infestation among goldfish with a χ^2 value of 34.88 and a *p*-value of <0.01. The prevalence and level of *A. japonicus* infestation among goldfish followed a normal distribution curve. The highest level of *A. japonicus* infestation among goldfish was recorded in the 5 to <10 category, where 59 (18.44%; 95% CI = 14.57–23.05) goldfish were infested with the range of *A. japonicus*. The lowest level of

A. japonicus infestation was observed in 14 (4.38%; 95% CI = 2.62–7.21) goldfish infested with <5 *A. japonicus*. The number of goldfish infested with *A. japonicus* in the other categories of the level of infestation ranged between those of 20 to 25 numbers of *A. japonicus* (9.69%; 95% CI = 6.91–13.42) and those of 10 to <15 numbers of *A. japonicus* (15.00%; 95% CI = 11.50–19.33) (Table 2).

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		

¥ = Significant at p <0.05

Infestation of *A. japonicus* on the skin of goldfish (*C. auratus*) showed histological lesions such as hyperplasia of goblet cells, scalp formation and edema of the skin, Zenker necrosis of the muscular layer of the skin, myositis, hemorrhage, and actenii arrows of scalps embedded in the dermis of the skin (Fig. 1).

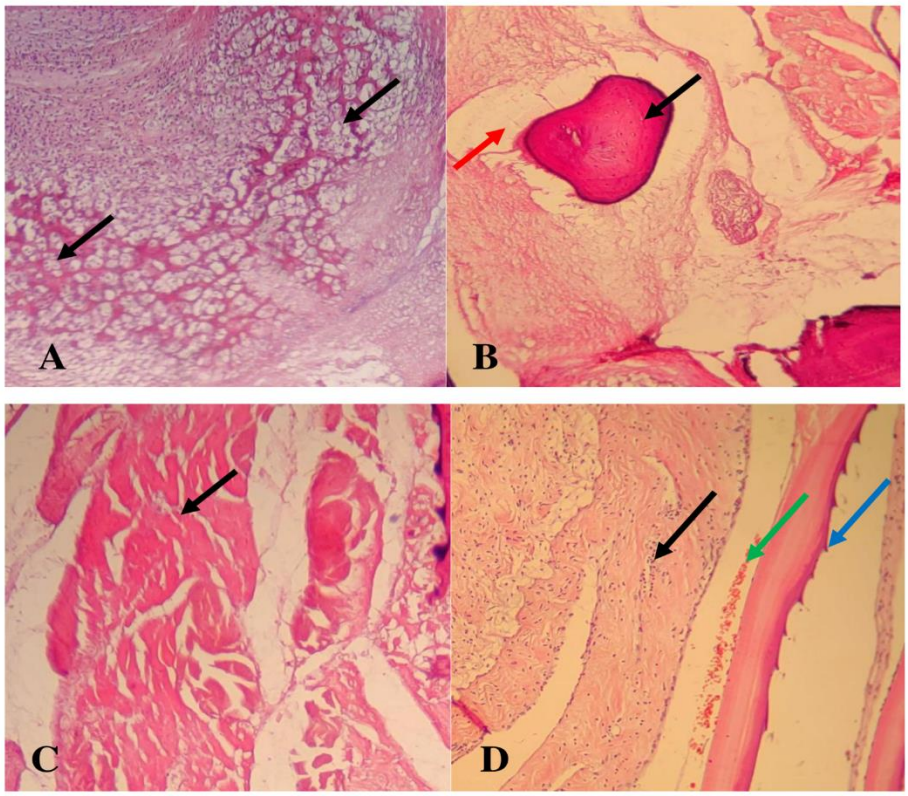

Figure 1.
Histopathological examination of goldfish (*C. auratus*) skin infested with *A. japonicus*; **1a**, showing hyperplasia of goblet cells (black arrows). **1b**, showing scalp (black arrow) and edema (red arrow). **1c**, showing Zenker necrosis of the muscular layer (black arrow). **1d**, shows myositis (black arrow), hemorrhage (green arrow), and actenii arrows of scalps 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).

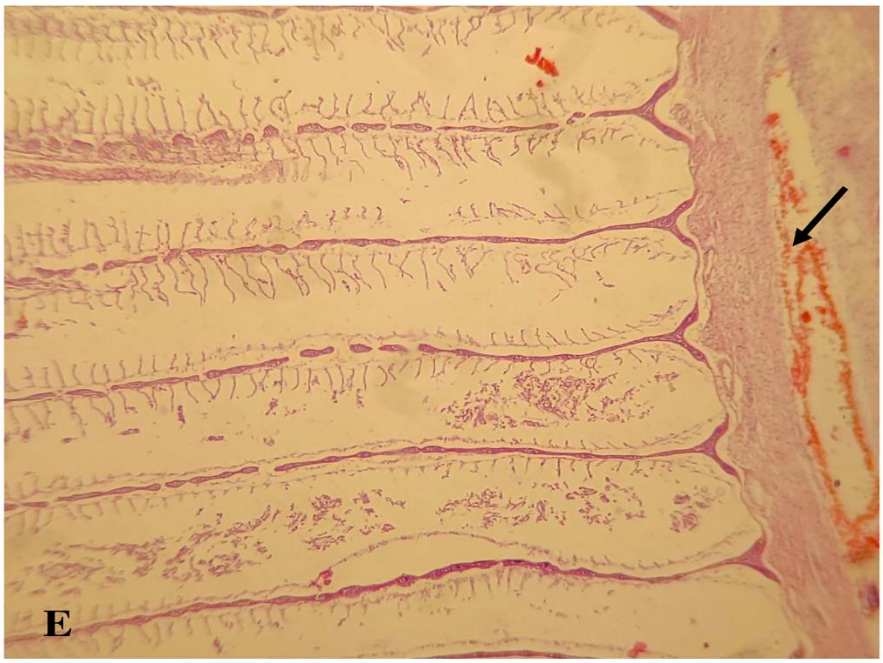

Figure 2.
Histopathological examination of goldfish (*C. auratus*) gills infested with *A. japonicus*; **2e**, showing hemorrhage (black arrow) and necrosis of gill filaments, H&E X40.

Discussion

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

In tandem with the high mean intensity and abundance of *A. japonicus* infestation recorded among goldfish in our study, Aalberg et al. [12] and Kismiyati et al. [11] also documented a high mean intensity and abundance of *A. japonicus* in fishes in Indonesia and Slovakia, respectively. This high 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 (as shown by the mean intensity and abundance) of *A. japonicus* in this study indicate that this fish louse is a generalist parasite, as postulated by many authors who reported a high prevalence and heavy infestation rate of the fish louse [9].

The heavy infestation with *A. japonicus* on the skin of goldfish in this study led to variable histopathological alterations in the musculoskeletal system, which are characterized by hyperplasia of goblet cells, scalp formation and edema of the skin, Zenker necrosis of the muscular layer of the skin, myositis, hemorrhage, and actenii arrows of scalps embedded in the dermis of the skin. 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], while Ahamad et al. [16] reported similar histological findings to Al-Darwesh et al. [15] in *Labeorohita* and *Cirrhinus mrigala* fishes.

The infestation of *A. japonicus* 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 irritating behaviour of fish, the sudden swimming with feeding regimes, and the *Argulus* louse attachment to the fish [18].

The penetration of the fish skin, muscles, and gills with the pre-oral stylet (modified mouthparts) of *Argulus* species results to hemorrhagic and erythema of the affected area. Numerous numbers of *Argulus* species feeding close to each other may cause localized swelling and edema of affected tissues. Inflammatory lesions that are characterized by hemorrhages, increased mucous secretion, and necrosis of the affected area may be due to the constant piercing of these lice with their stylet and the inoculation of cytotoxic enzymes [19, 20]. 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 infested fish [21]. These histopathological alterations may have resulted from the appendages and hooks of *A. japonicus* penetrating the skin and muscle and the insertion of their stylet (the mouthpart adopted for sucking blood) [22].

In summary, our findings reveal that *A. japonicus* infestation was prevalent (61.56%) among goldfish (*C. auratus*) in Mosul, Iraq, and the infestation of the fish louse was high. The infestation of this fish louse caused diverse histological lesions on the skin and gills of the infested goldfish.

Materials and methods

Study area

This present study was carried out in Mosul; the city is situated on the west bank of the Tigris, opposite the ancient Assyrian city of Nineveh on the east bank [23]. Mosul sits at 43.09 E and 36.19 N, at 230 meters above sea level, and it covers a total land area of 70 sq mi (180 km^2). The city has a climate that is characterized by cold winters with occasional snow and dry and hot summers [24].

Goldfish collection

Three hundred and twenty goldfish were purchased from fish markets and fish stores in the city of Mosul, Iraq. The purchased 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 further parasitological and pathological studies.

Collection and parasitological identification of *Argulus japonicus*

Argulus japonicus was collected manually from the gills, fins, operculum, and entire skin of the fish with the aid of fine forceps following a thorough and careful external examination of the fish.

Argulus japonicus from each goldfish was collected separately into sample bottles containing 70% ethanol to morphologically identify the ectoparasites and count and record the intensity of their infestation. The collected *A. japonicus* were identified with the aid of a stereomicroscope using entomological keys, as documented by Bykhovskaya-Pavlovskaya et al. [25] and Rushton-Mellor [26].

Histopathological analysis

Few of the goldfish infested with *A. japonicus* were anesthetized, as described by AL-Taee et al. [27]. Muscle, skin, and gill samples were collected and fixed in neutral buffered formalin (10%) for a minimum of two days. Afterward, the fixed tissue sections were cut into small parts of about two to three mm thickness and then dehydrated using 70%, 80%, 90%, and 100% (ascending grades) of alcohol for fifteen minutes, and by clearing in absolute (100%) xylene and embedding in paraffin wax. Sections of five-micron thickness were cut and stained with H&E as described by Akanbi et al. [28]. Lastly, the slides stained with hematoxylin and eosin were examined at X40 magnification using a compound microscope.

Statistical evaluation

The prevalence, abundance, and mean intensity of *A. japonicus* infestation in goldfish were calculated using the ecological formulae (in terms of comparative parameters) for parasite infestation as documented by Margolis et al. [29] and Bush et al. [30].

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

$$\text{Mean Intensity} = \frac{\text{Number of collected parasite(s)}}{\text{Number of fish infected}}$$

$$\text{Abundance} = \frac{\text{Number of parasites(s)}}{\text{Number of fish examined}}$$

The 95% CI was calculated with respect to the prevalence. Statistical significance was set at $p<0.05$.

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.

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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Figure legends

Figure 1.

Histopathological examination of goldfish (*C. auratus*) skin infested with *A. japonicus*; **1a**, showing hyperplasia of goblet cells (black arrows). **1b**, showing scalp (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 actenii arrows of scalps embedded in the dermis (blue arrow) H&E X40.

Figure 2.

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