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Case Report

Fish Lice (*Argulus japonicus*) in Goldfish (*Carassius auratus*)

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Fish lice (*Argulus* spp; family Argulidae) are branchiuran crustaceans that parasitize both marine and freshwater fishes. *Argulus* spp can be a major threat to fish health, because heavy infestations can cause significant morbidity and mortality. In addition, fish lice are known to be the vehicle for other fish diseases. During rounds at our facility, *Argulus japonicus* was collected from the caudal and anal fins of 3 goldfish (*Carassius auratus*).These goldfish were asymptomatic, and no additional cases were noted after manual removal of the lice. As soon as any *Argulus* organisms are identified, management and treatment are recommended because infections can escalate rapidly. Currently, there are no FDA-approved drugs for the control and treatment of this parasite, but several chemicals including organophosphates and diflubenzuron have been used with success. The screening and quarantine of incoming fish is the best way to avoid a facility-wide *Argulus* infestation.

Fish lice (*Argulus* spp) are members of a large group of branchiuran crustaceans that infest and cause disease in fish. More than 100 different species of *Argulus* are distributed worldwide.[12] The 3 most studied species—*Argulus foliaceus*, *A. japonicus*, and *A. coregoni*—are found in freshwater systems.[12] *Argulus* lice have compound eyes, a suctorial proboscis, 2 anteroventral prominent sucking discs that serve as attachment organs, 4 pairs of thoracic swimming legs, and a laterally expanded carapace that forms respiratory alae.[2]

Argulus japonicus is native to Asia, where its typical hosts, goldfish (*Carassius auratus*) and common koi carp (*Cyprinus carpio*) are also located.[6] In the United States, *A. japonicus* is present in Florida, Georgia, Louisiana, California, Hawaii, Illinois, Maryland, Washington, and Wisconsin.[6] It is suspected that *A. japonicus* has been transported around the world unintentionally, with stocks of ornamental fish, particularly koi carp.[6] During rounds at our facility, the branchiuran parasite *A. japonicus* was collected from the caudal and anal fins of 3 goldfish (*Carassius auratus*).

Argulus spp can be a major threat to fish health. Heavy infestations of this parasite can cause significant morbidity and mortality.[3,6] In addition, *Argulus* lice have been known to be the vehicle for other fish pathogens, including *Rhabdovirus carpio*, larval nematodes, and the fungus *Saprolegnia*.[1,4] Fish without visible lice may show nonspecific signs of infestation, including spot or pinpoint hemorrhages, anemia, fin and scale loss, increased mucus production, lethargy, erratic swimming, and poor body condition.[12] Fish may rub against surfaces in an attempt to relieve irritation or to remove the parasites.[12] In some cases, there may be no obvious signs of disease other than presence of the parasite.[12]

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Case Report

A shipment of goldfish (approximate age, 2 y; $n = 60$) was received at our facility (The University of Texas Health Science Center at Houston, Texas) from a commercial supplier and assigned to an IACUC approved protocol. The room into which the fish were received contained 2 self-contained, recirculating, aquatic rack systems, one to supply fish for research and the other to hold incoming fish. These systems each comprise six 20- to 30-gallon tanks using a multistage filtration process and UV sterilization of filtered water. The filters include a mechanical-fiber pad, a fluidized bed biofilter, and a mechanical–chemical fiber sleeve with carbon. Water temperature is maintained at 20 to 22 °C by maintaining the room temperature at the appropriate level. The room is on a 12:12 h light:dark cycle. All new arrivals are quarantined for at least 3 wk. When the research tank is depopulated, the fish in the quarantine system are used for research, and new fish are received into the previous research system.

Approximately 2 mo after the goldfish had arrived and while they were in the quarantine system, a 'shadow' on the caudal fin of one of the fish was observed during a routine husbandry check. Gross examination of the fish revealed that the shadow was a live organism resembling a fish louse. This organism was removed with forceps and placed in a beaker of tank water. Manual inspection of the other 8 fish cohoused with the index fish disclosed lice on the anal fins of 2 additional goldfish (Figure 1). These parasites were removed and placed in the beaker with the first specimen. All remaining fish in the system were inspected visually, and no other parasites were seen. The infected goldfish parasitized with lice did not demonstrate any clinical signs and therefore were placed back into tanks. A pathologist confirmed the organism to be *A. japonicus* (Figure 1). Because our facility did not have a severe outbreak, and only a few fish were affected, we opted for the conservative approach of manual removal of lice without further medical intervention. After the lice had been removed, all goldfish

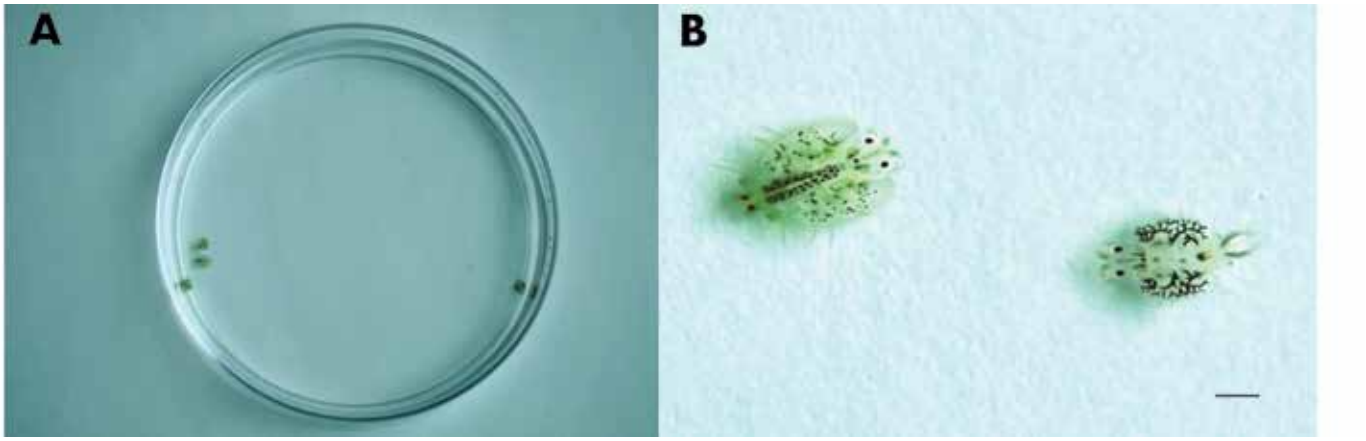

Figure 1. (A) The branchiuran parasite *Argulus japonicus* collected from the caudal and anal fins of 3 goldfish (*Carassius auratus*). (B) *Argulus japonicus*. Bar, 1 mm.

in the affected system were monitored daily for evidence of adult *Argulus*; 10 mo later, no more fish lice were noted.

Discussion

Although rarely diagnosed in laboratory animal facilities, *Argulus* spp are an important parasite in aquatic colonies and warrant additional study. These organisms have a direct life cycle (requiring a single host), and *A. japonicus* are dioecious, meaning that sexually distinct male and female forms occur.[6,12] Female *Argulus* lice have small spermathecae, and male lice have large testes, which are visible in live specimens due to the transparent properties of this parasite.[3] Mating usually occurs on the epithelial surface of the host fish.[6] Female lice then detach from the host and lay 1 to 9 strings of 5 to 226 eggs, which are covered in gelatinous excretions, in 1 to 6 rows on hard substrates.[12] The eggs of *A. japonicus* hatch in 10 d at 35 °C but require 61 d to hatch at 15 °C.[12] Newly hatched larvae survive for 1 to 2 d on nutrition from their yolk sacs but thereafter require nutrition from the host's epithelial cells and mucus.[1] *Argulus* lice propel themselves through water very efficiently when they seek a new host; propulsion is provided by the 4 pair of thoracopods located on the posterior portion of their ventral surface. On the ventral surface of the louse head are short antennules and antennae, both of which have claws and function as organs of attachment to the host. Juvenile *Argulus* lice attach to the host by means of the claws, whereas adults attach by means of a pair of modified suckers commonly referred to as maxillules.[3] The majority of the *Argulus* life cycle takes place on the body of the host fish in 30 to 100 d.[6] In particular, *Argulus japonicus* continuously lays eggs, which may stay dormant until temperatures conducive to hatching occur in spring.[6] As this species develops, it passes through approximately 6 larval instar stages before metamorphosing into the adult form. Adults can survive without a host for approximately 2 wk.[1,5,10-13]

While feeding on the host, *Argulus* species insert a preoral stinger to inject digestive enzymes into the body of the fish, and the lice suck out liquefied body fluids by using their proboscis-like mouth. Feeding occurs on the skin or gills of the fish, causing intense irritation and tissue damage.[7,15] Pathology caused by *Argulus* is related to the trauma induced by the attachment and feeding methods, and areas where feeding has taken place usually result in hemorrhagic spots. Lesions inflicted by the parasites often become necrotic and ulcerated, leading to secondary infections.[9]

Under low magnification, *Argulus*-associated lesions appear as craters, which form due to hyperplasia of the epidermis at the margins of the wound.[14] The craters may be restricted to the epidermis, especially on large fish that have a thick epidermis. Mucus and club cells are absent from any epidermis remaining in the crater but are abundant in the tissue at the margin of the crater. In addition, this parasite can penetrate through to the stratum spongiosum of the dermis and even to the stratum compactum beneath; consequently the dermis becomes edematous.[14] In terminal cases, the entire epithelium of the host becomes thin and may be missing from parts of the body and fins.[14]

Due to their size, older stages of *Argulus* can be diagnosed with the naked eye and are visible moving on the host or swimming in the water. In addition, the parasite can be identified on wet mounts of affected tissue. Captured fish should be examined quickly because *Argulus* lice can leave the fish rapidly once it is disturbed or removed from the water.[12] Differentiation of *A. japonicus* from other species such as *A. foliaceus* and *A. coregoni* is based on their physical appearance. *A. foliaceus* has rounded abdominal lobes, the posterior emargination does not reach the midline, and the posterior lobes of the cephalothoracic carapace do not extended beyond the beginning of abdomen. In contrast, *A. coregoni* has pointed abdominal lobes and a body length of 12 mm, whereas the posterior lobes of the cephalothoracic carapace of *A. japonicus* extend beyond the beginning of abdomen. In addition, the lobes are more pointed in *A. japonicus* than in *A. foliceus*.[7,15]

As soon as *Argulus* is identified, management and treatment are recommended due to the potentially rapid escalation of infection. There are currently no FDA-approved drugs for the treatment and control of *Argulus*. The most effective treatment against argulusosis is organophosphates, which usually are given as 2 or 3 doses at 1-wk intervals to kill emerging larvae and juveniles.[7] Because adult fish lice continue to molt, they are susceptible to chitin synthesis inhibitors, such as the pesticide diflubenzuron.[8] Diflubenzuron (Dimilin, Chemtura) interferes with the molting of the parasite's exoskeleton during growth and development, effectively killing both adult and larval stages, but it is a restricted-use pesticide. Other compounds with a similar mechanism of action as that of diflubenzuron, such as lufenuron (0.13 mg/L; Program, Novartis Animal Health), have also been used with success.[12] Oral enamectin is also effective. Other treatments of Argulus infestations include the use of common chemicals such as salt (NaCl), formaldehyde, potas-

sium permanganate (2-5mg/l bath), and formalin[7] Two applications of trichlorfon (0.25 ppm; Masoten, Dylox) 1 wk apart can be used as well.[2] To eradicate *Argulus*, treatments such as trichlorfon (0.25 ppm for several hours) and emamectin benzoate have been used.[7] It can take about 2 mo for *Argulus* to complete their life cycle; therefore, it is useful to rid tanks of egg contamination by using a disinfectant or by allowing the tanks to dry thoroughly for several days.[8] Manual removal, as we have done at our facility, can be effective also.

To avoid a facility-wide *Argulus* infestation, screening and quarantine of incoming fish are very important and recommended. Incoming fish, particularly wild-caught or pond-raised stock, should be quarantined, observed, and sampled to minimize the risk of introduction. Source water should be evaluated to ensure that it is not a route for the introduction of argulid eggs.[12] In rooms with multiple users, each tank has a designated net to reduce possible transmission of parasites. After each use, nets are disinfected in a solution of 2 ounces of 6% bleach in 1 gallon of tap water. This is the first report of *Argulus japonicas* at the University of Texas Health Science Center at Houston.

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