RESEARCH

THE USE OF LUFENURON TO TREAT FISH LICE (*ARGULUS* SP) IN KOI (*CYPRINUS CARPIO*)

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Abstract

Fish lice can cause significant morbidity and death in heavily infested fish. In addition to being a mechanical irritant when sucking blood, *Argulus* sp have been shown to be the vector for other fish diseases. Koi carp from a pond environment were presented with multiple raised dark spots on their dorsa and sides. The primary differential disease diagnosis was an *Argulus* sp. Parasites removed from the affected fish were examined under a dissection microscope, and the definitive diagnosis was determined to be *Argulus* sp infestation. Treatment with lufenuron (Program; Novartis, Greensboro, NC USA) was initiated with one 409.8-mg tablet per 1,000 US gallons (3,785 L) of water for a concentration of approximately 0.1 mg/L. This treatment was repeated once per week for 5 weeks. The fish have been monitored for 13 months after the initial treatment, and thus far, there has been no reappearance of *Argulus* sp within the aquatic environment.Published by Elsevier Inc.

Key words: *Argulus*; *Cyprinus carpio*; fish louse; koi; lufenuron

Fish lice can cause significant morbidity and death in heavily infested fish. In addition to being a mechanical irritant when sucking blood, *Argulus* sp have been shown to serve as a vector for other fish diseases. Spring viremia of carp (SVC), a fatal disease of cyprinid fish, has been documented to transfer between hosts by *Argulus* sp, which acts as a mechanical vector for SVC virus.[1] In the past, SVC outbreaks have resulted in significant economic losses in the United States.[2]

Argulus is a crustacean parasite in the subphylum *Crustacea* and is in the same taxonomic group as prawns, shrimp, and water fleas. All animals in this group have a semi-rigid to rigid chitin exoskeleton, and as with many other ectoparasites, *Argulus* lice will molt this exoskeleton when growing. Lufenuron, a benzoylurea pesticide, inhibits the chitin biosynthesis. Without chitin, aquatic parasites like *Argulus* sp will be unable to form an exoskeleton, thereby preventing the ectoparasites from developing into adults. The use of lufenuron to treat ectoparasites in freshwater fish has been reported in the literature based on anecdotal evidence, but no scientific studies of its use have been reported to date. This case report describes the successful use of lufenuron in the treatment of koi (*Cyprinus carpio*) infested with fish lice (*Argulus* sp) in a pond.

The owner of a koi pond contacted the Zoological Medicine Service at the University of Georgia College of Veterinary Medicine (Athens, GA USA) and reported that progressive dark brown spots had developed in koi from 1 pond on the property on their tails, fins, and bodies over a 14-day period. The owner initially observed the questionable spots on a few fish, but

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when the university was contacted, all 12 fish in the 3,800-L (1,000–US gallon) pond were affected. The fish in this pond were approximately 8 years old and had been acquired from various pet stores. Approximately 3 months earlier, the owner transferred 3 juvenile koi from another pond on the same property to the affected pond.

There was no previous history of disease in the pond with the affected fish. Well water was used to maintain this pond, and one-third of this water volume was changed every 3 to 4 days to maintain adequate water quality. The water quality protocol, filtration system, and water fall for aeration were in place because there were no live plants in the pond because the fish had uprooted and destroyed plants in the past. At the time of the consultation, the owner did not test the water quality levels on a regular basis. The water quality analysis conducted during the consultation visit showed values compatible with normal parameters: pH, 7.0; total ammonia nitrogen, 0.2 ppm; and nitrite, 0.05 ppm. The fish were fed a commercial floating fish pellet product, and the owner set up feeding to provide enough pellets to last for 5 minutes of feeding twice a day. Although the water temperature of the pond was not measured with a thermometer, the water was not found to be subjectively warm during the water chemistry tests. The site visit took place in May, and the water in the pond came from an underground well. In addition, the koi did not show any clinical signs associated with hypoxia (e.g., gilling, piping, surface air sucking), which are often present if dissolved oxygen levels are low. Supplemental aeration is often required to counteract low dissolved oxygen levels when the water temperature rises above 26.6°C (80°F). As a routine, the owner always implemented a quarantine period for newly acquired fish before placing them in established ponds. The quarantine protocol consisted of a 1-month isolation period in a separate pond.

FIGURE 1. The ectoparasite (*Argulus* sp) (arrow) attached to a fish at the time of the initial physical examination.

On initial observation, the fish had multiple raised dark spots on their bodies. Two fish were then caught with a net and placed into a 38-L (10–US gallon) anesthesia tank for closer examination. Both fish displayed a number of these dark spots. Anesthesia was induced with tricaine methanesulfonate (100 mg/L, buffered with sodium bicarbonate [$NaHCO_3$] at 200 mg/L [MS-222]) (Finquel; Argent Chemical Laboratories, Inc., Redmond, WA USA). The fish were examined immediately, and it was noted that both had multiple ectoparasites on their dorsa and fins (Fig. 1). *Argulus* sp infestation was considered the primary differential disease diagnosis for the presenting complaint. No other abnormalities were noted during the initial external physical examination.

Some of the parasites were manually removed from the fish and brought back to the veterinary teaching hospital for confirmation of the preliminary diagnosis. The parasites were examined under a dissection microscope and showed the anatomic structures typical of *Argulus* sp (Fig. 2). An exact species identification was not considered necessary to develop an adequate treatment plan.

Treatment with lufenuron (409.8-mg tablet per 1,000 US gallons [3,785 L] of water, which produced a final concentration of approximately 0.1 mg/L, once a week for 5 weeks) (Program; Novartis, Greensboro, NC USA) was initiated.[3] The owner was instructed to completely crush the tablet into a powder before adding it to the system close to the waterfall to ensure even distribution of the chemical. The lufenuron treatment was repeated after new water was added.

The owner reported that approximately 4 days after the first treatment, the parasites on the koi had significantly decreased in number and there were no obvious adverse side effects to the fish. Approximately 10 days after the initial treatment, the owner reported that no parasites were evident on the fish, even when the koi were closely examined. No ectoparasites were found on the fish during the follow-up consultation visit to the pond approximately 30 days after the initiation of treatment. More than 1 year after treatment, the koi collection is considered free of *Argulus* sp based on continued examination and strict monitoring for recurrence.

FIGURE 2. Light microscopy appearance of *Argulus* sp. One should note the suction cups (thin arrow) and eyespots (thick arrow). Original magnification was ×10.

DISCUSSION

Fish lice of the genus *Argulus* are found worldwide, apart from the polar regions.[4] They were thought to only infect fish, but they have been shown also to infect frogs.[5,6] *Argulus* sp are also a commonly reported problem for the fishpond hobbyist, and more importantly, they have also been involved in large economic losses such as those associated with SVC, where the parasites act as the major biological vector of this disease.[7] Fish lice infections are not limited to the freshwater environment. In 1995 it was estimated that infestations caused by another ectoparasite, the sea louse (*Lepeophtheirus salmonis*), which is a distant relative to *Argulus* sp, resulted in approximately $20 million in losses to the New Brunswick fishing industry.[8]

Fish lice are some of the largest ectoparasites of fish and can be observed with the naked eye. *Argulus foliaceus* ranges from 5 to 10 mm in length; given the relatively large size, the lice can be easily recognized, as was found in this case.[9] On infested fish, one will see small dark spots and may not realize that they are in fact ectoparasites until movement is noted. *Argulus* sp are often located behind the fins or around the head and are easier to visualize on the fins because of the backlight through the thin tissue membrane. These parasites have a direct lifecycle.

In addition to living on the host, *Argulus* sp spend a significant amount of time swimming freely during the mating process. After mating, the egg cluster is dropped by the female on a submerged structure (e.g., rock). Bower-Shore[10] described the lifecycle of *A foliaceus* and stated that the eggs take 30 to 35 days to hatch at an undisclosed temperature. After hatching, the larvae must find a host for a blood meal within 4 days to ensure survival. The larvae go through several metamorphic changes before reaching maturity, with the entire lifecycle taking between 30 and 100 days depending on the water temperature. The parasite can overwinter either as larval stages or in dormant eggs.

Severely affected fish exhibit patches of hemorrhagic and edematous skin or fins. The resulting dermatitis associated with *Argulus* sp infestations is caused by the parasite attaching to the fish with its curved hooks and sucker.[4] The rim of the sucker compresses epithelial cells in the shape of a ring, whereas the vacuum action of the sucker causes the elevation and loss of cohesion of cells in the center of the ring.[4] The feeding apparatus of the parasite further injures the fish when a stylet is inserted through the epidermis and into the underlying host tissue for a blood meal, during which enzymes and digestive chemicals are released, causing irritation, discomfort, and hemorrhage. These lesions are predisposed to secondary bacterial infections.[9] In the rainbow trout (*Oncorhynchus mykiss*), it has been established that there is a link between *Argulus coregoni* infection and susceptibility to bacterial disease.[11] It is during the blood meals that the host fish can be exposed to other pathogens.[7] Anemia is another significant pathologic effect associated with feeding, and Hindle[12] also reports that fish infested with *Argulus* sp were lethargic and isolated themselves in the corners of aquariums. In addition to these direct pathogenic effects, *Argulus* sp are also known to be a vector of certain viruses, such as SVC virus and carp pox (cyprinid herpesvirus 1).[4]

Historically, eradication of *Argulus* sp infestations has been challenging despite use of chemical treatments (e.g., salt [sodium chloride], formaldehyde, potassium permanganate, formalin, trichlorfon, emamectin benzoate, powdered quicklime).[3,4,13] However, many of these chemical compounds are unapproved, hazardous to the operator and the fish, or simply ineffective. Ivermectin has been used as an oral treatment, although it is not registered for food fish and is only available through an extra-label veterinary prescription.[5] Another member of the avermectin family, emamectin (Slice; Schering Plough, Kenilworth, NJ USA), which is a licensed oral treatment for lice in salmonids, has recently been evaluated for control of freshwater *Argulus* sp in goldfish (*Carassius auratus auratus*) and koi.[14] Emamectin is administered in top-dressed, pelleted commercial fish feed and has been found to be very effective in treating affected fish. The goldfish were fed the medicated diet (50 μg/kg body weight per day) for 7 consecutive days. After treatment, no lice were found on the goldfish in the treated group. In a separate experiment, affected koi were fed a low-dose medicated diet (5 μg/kg body weight per day) for 7 days. After treatment, no lice were found on the koi.[14]

A different class of drug has also been used to effectively kill arthropod ectoparasites in a commercial fish farm environment. Teflubenzuron is an insecticide of the benzamide class; it works as an orally administered chitin synthesis inhibitor and has been shown to be effective against all stages of sea lice that undergo molting, including the larval and pre-adult stages.[15] The treatment of ectoparasites with teflubenzuron has been described by the oral route but not as a water treatment for a closed aquatic system. Teflubenzuron is approved as an in-feed treatment for sea lice control in Norway, the United Kingdom, and Ireland.[8] Teflubenzuron is part of a group of compounds called the acylureas (benzophenylureas), which act to disrupt the synthesis of chitin, a polysaccharide of particular importance to arthropods.[16] An oral dose of teflubenzuron—10 mg of active ingredient per kilogram of biomass daily for 7 days—was administered to Atlantic salmon (*Salmo salar*).[7] The concentration of the teflubenzuron in the medicated feed for the Atlantic salmon was 2 kg per metric ton.[8] To our knowledge, no report exists of a water treatment regimen with this drug class.

Lufenuron is a related drug that affects the parts of the arthropod lifecycle where chitin is being formed (i.e., larval and pre-adult stages in sea lice) and where its incorrect or insufficient production can lead to malformations of the exoskeleton.[9] Regarding the safety of lufenuron, the following values have been established for the median lethal dose of fish: LC_{50} (lethal concentration, 50%; 96 hours) greater than 73 mg/L for rainbow trout, greater than 63 mg/L for carp, greater than 29 mg/L for bluegill sunfish, and greater than 45 mg/L for catfish.[17] The frequency of the lufenuron treatment is mainly governed by the pH of the affected aquatic system. Data for the degradation half-life of lufenuron in water are 32 days at pH 9.0, 70 days at pH 7.0, and 160 days at pH 5.0.[16] The optimal pH for a koi pond is 7.0 to 7.4; it was 7.0 in this case. The owner performed regular partial water changes (30% of total volume) once a week because of a concern about the water quality. Because of this dilution effect of the water on the drug concentration, the owner was instructed to perform a complete water change once a week for 5 weeks and add a full dose of lufenuron immediately after each change.

In biologically active soils, under aerobic conditions, lufenuron is rapidly degraded and the degradation half-life is 13 to 20 days.[17] This is an important factor to consider because runoff or spillage of treated pond water can have a negative impact on many crustaceans within the surrounding environment. Lufenuron is not classified as a dangerous substance to humans if it is swallowed, is inhaled, or makes contact with the skin. However, lufenuron is classified as sensitizing,[17] which is defined as an allergic reaction to a particular irritant that results in the development of skin inflammation and itchiness. Unlike normal skin irritation, the sensitized skin becomes increasingly reactive to the substance as a result of subsequent exposures.[7]

Toxicity studies have been performed on different classes of animals, and testing indicated a low acute, short-term, and long-term toxicity to birds; low acute and chronic toxicity to fish and algae; high acute and chronic toxicity to aquatic invertebrates; and low acute toxicity to honeybees and earthworms.[17]

This is the first report in the scientific literature describing the effectiveness of lufenuron-treated water in eliminating *Argulus* sp from a pond. Caution must be observed regarding environmental contamination of lufenuron-treated water because of the potential harmful impact of wastewater on wild crustaceans living in the surrounding environment. This case demonstrates the successful use of lufenuron to treat pond fish diagnosed with *Argulus* sp.

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