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Case Report

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First reported case of Chironomid midge larva infestations in rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) eggs

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Abstract: Six days after transfer of eyed stage rainbow trout (*Oncorhynchus mykiss)* eggs into an incubator, egg mortality occurred during the winter of 2007. During the outbreak, approximately 30% of the eggs died. *Rheocricotopus fuscipes* (Kieffer, 1909) and *Eukiefferiella claripennis* (Lundbeck, 1898) (subfamily *Orthocladiinae*), Chironomid midge larvae were observed on the eggs by examining the eggs under the microscope. Histological sections of the infested eggs exhibited midge bite marks. This is the first report of Chironomid midges preying on fish eggs.

Key words: Fish eggs, *Rheocricotopus fuscipes, Eukiefferiella claripennis*

Gökkuşağı alabalığı (*Oncorhynchus mykiss* Walbaum 1792) yumurtalarında meydana gelen Chironomid sinek larva enfestasyonu

Özet: Bu çalışmada, 2007 yılında gözlenmiş gökkuşağı alabalığı (*Oncorhynchus mykiss)* yumurtalarında yaklaşık % 30 oranında meydana gelen ölümlerin sebebi araştırıldı. Mikroskop altında yapılan incelemelerde, yumurtaların üzerinde *Rheocricotopus fuscipes* ve *Eukiefferiella claripennis* (alt aile *Orthocladiinae*) olmak üzere iki farklı Chironomid sinek larvası gözlemlendi. Histolojik olarak yapılan incelemede sinek larvalarının yumurtalara zarar verdiği belirlendi. Bu çalışma ile Chironomid sinek larvalarının balık yumurtaları ile beslendiği ve yumurtalarda ölümler meydana getirdiği ortaya konulmuştur.

Anahtar sözcükler: Balık yumurtası, *Rheocricotopus fuscipes, Eukiefferiella claripennis*

Introduction

Insects of the family *Chironomidae* (Insecta: Diptera), known as midges, are often the most abundant insects inhabiting freshwater environments (1-4). Midges are fragile and mosquito-like in appearance, but they do not bite. They are the key members of stream macro-invertebrates assemblages, playing an important role in detritus processing and food chains (5). Chironomids are opportunistic parasites and rapid colonizers that quickly adapt to fluctuating conditions (5). More than 10,000 species of *Chironomidae* exist (2). These aquatic insects are

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tolerant of a wide range of environmental conditions such as swift moving streams, deep slow moving rivers, stagnant ditches, and in lakes and ponds that are rich in decomposing organic matter (2).

Developing larvae consume suspended organic matters in the water and in the mud; therefore, larvae clean the aquatic environment by recycling organic debris. The young midge larvae eat dead plant and animal materials (detritus) at the bottom of the water. They also eat bacteria in the mud and help dispose of human waste in sewage plants. Chironomid midges are a major component of the diet of many fish species. In particular, bottom-feeding fishes, such as catfish and carp, consume large numbers of midge larvae (6,7). Chironomid species are consumed in the hatchery ponds by the young trout after they hatch. Although such a relationship is very well documented in the literature (8), a parasitism of Chironomid midge larvae in rainbow trout eggs has not been documented yet. In the present study, preying of midges on fish eggs was described.

Case history

Eyed stage eggs were obtained from a commercial farm. When eggs brought to the hatchery in January 2007, eggs were disinfected with 100 mg/L iodine (polyvinylpyrrolidone iodine; Sigma-Aldrich Corporation, St Louis, Missouri, USA) for 10 min. Then the eggs were rinsed with clean water to remove iodine residues before putting them into a vertical tray incubator for hatching.

Six days after transferring fish eggs into the incubator, some eyed stage eggs turned into white. Upon examining the eggs under a microscope, we found midges that were attached to the surface of the eggs. On the consecutive days, eggs mortality was increased. Water had the following characteristics following the infestation of the eggs with midges: dissolved oxygen 9.00 ± 0.25 mg/L, temperature 14 ± 2 °C, pH 6.80 ± 0.17, and total hardness 45 ± 2 mg/L as $CaCO_3$.

Each of 10 fully grown larvae (with the anterior segments expanded), pupae, and male adult midges were preserved in 70% ethanol. Slide mounted single exuviae of adult males were examined under the microscope (9).

Ten Chironomid midges attached to eggs were removed from the incubator and preserved in 10% neutral buffered formalin (NBF) for 48 h. Organs were rinsed in 4 changes of 70% ethanol (EtOH), and stored in 70% EtOH until further processing. They were dehydrated in isopropanol, cleared in xylene, infiltrated in paraffin, and sectioned at a thickness of 15 µm. Since fish eggs are very fragile, they were kept in liquid paraffin for 24 h. Teeth marks of the midges on the eggs were examined with a light microscope.

Infected fish eggs were treated with formaldehyde (100, 200, and 300 mg/L for 40 min), copper sulfate (0.5 and 5 g/L for 30 min and 5 min, respectively), hydrogen peroxide (100 and 1000 mg/L for 40 min), and dichlorvos (dimethyl 2,2-dichlorovinylphosphate; 0.02, 0.55, and 1.1 g/L for 10, 2, and 1 min, respectively).

Results and discussion

In a rainbow trout hatchery, 6 days after transferring eyed stage fish eggs into the incubator, 30% eggs mortality occurred during the winter of 2007. To find the cause of mortality, live eggs were examined under a microscope and consequently midge larvae was found, attached to the surface of the eggs (Figure 1). After examining the cut surface of the trout eggs, we observed teeth marks of midge on the integument of the eggs (Figure 2).

The adult male with associated pupal exuviae and larvae (with bluish body color and dark brown head capsule) was identified as *Eukiefferiella claripennis*. Most of the larvae with yellow-brown head capsules and partially reddish thorax segments belong to *Rheocricotopus fuscipes*.

After leaving the egg mass, midge larvae came into hatchery with water and attached on the fish eggs. After they grow, the larvae take on a light brown color and consequently they turned into mature larvae. The larval stage took less than 10 days at 14 °C water temperature. Larvae transformed into pupae while they are still in their tubes that are attached on the eggs. After 3 days, pupae actively swim on the surface, and subsequently adults emerge several hours later. Pupation and emergence of adults occurred in January when the average water temperature was 14 °C.

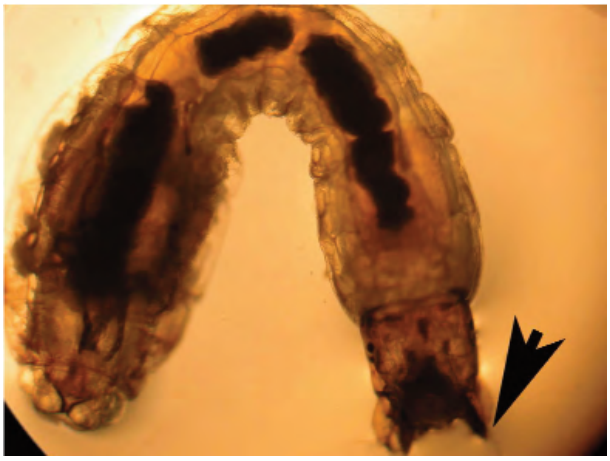

Figure 1. Penetration of Chironomid midge larvae to the eggs.

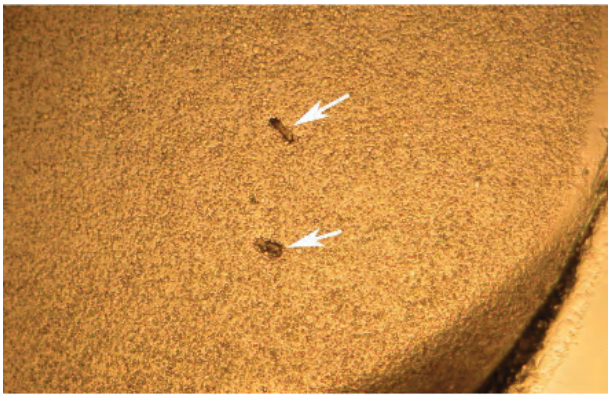

Figure 2. Teeth marks of the midges observed on the raw section of the egg integument.

Formaldehyde, copper sulfate, and hydrogen peroxide were not effective to treat Chironomid midges infestation. Dichlorvos was very effective even in low concentrations to treat the Chironomid midge larvae infestation.

The larvae of most midges are aquatic and feed primarily on algae and decaying organic matters and, in turn, are readily fed upon by fish (10). A few species are capable of mining the soft tissues of submersed plants and using the living plant material as a food source (1). However, the present study illustrates Chironomid midge larvae infestations on fish eggs. This is the first documented report of Chironomid midges parasitizing fish eggs.

The envelope surrounding the animal egg plays significant roles in reproductive and developmental processes such that it acts as an interface between the egg and sperm as well as an interface between the embryo and its environment (11). The egg envelope is a major structural determinant of the eggshell in fish, and is often referred to as zona radiata because of its striated appearance under the light microscope (12). The egg envelope provides physical protection from the environment and plays a role in diffusive exchange of gases (11). The egg envelop can be damaged and the yolk sac can be exposed to water and then eggs die following exposure to hazardous effects such as those caused by a midge bite.

Although there was a common belief that the mouthparts of these larvae do not fit to penetrate the integument of trout eggs, the present study clearly demonstrated that *Rheocricotopus fuscipes* and *Eukiefferiella claripennis* can penetrate into eggs as evidenced by the parasite bites in histological sections of eggs.

Acknowledgments

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