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Control of Predacious Flatworms *Macrostomum* sp. in Culturing Juvenile Freshwater Mussels

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Abstract.—Flatworms of the genus *Macrostomum* are voracious predators on newly metamorphosed juvenile freshwater mussels (Unionidae), which require a fish host to transform mussel larvae into free-living juveniles. Toxicity tests were performed with formalin (paracide-F, 37% formaldehyde) to determine the appropriate levels of treatment for eradicating these flatworms from host fish tanks without adversely affecting the culture of juvenile mussels. Results indicate that a 1-h shock treatment of 250 mg/L formalin or a 3-d continuous exposure to 20 mg/L of formalin kills adult *Macrostomum* but not fish. Observations indicate that a single treatment is insufficient to kill *Macrostomum* eggs, so a second treatment after 3 d is necessary to kill newly hatched flatworms. Newly metamorphosed freshwater mussels exposed to similar shock and continuous treatments of formalin were also killed. Thus, all host fish introduced for the purpose of mussel production should be quarantined and treated prophylactically to avoid the infestation of mussel culture systems with predacious flatworms.

Production of juvenile freshwater mussels (Unionidae) requires host fish to transform mussel larvae, or glochidia, into free-living juvenile mussels. Various fish species serve as hosts to different mussel species. Collection methods vary for the host species used but may include backpack or boat electrofishing, seining, and sometimes purchases from a hatchery.

Observations of flatworms in aquaculture holding tanks containing juvenile freshwater mussels confirm that they are significant predators of newly metamorphosed juvenile mussels. In the freshwater mussel propagation facility at the Aquaculture Center at Virginia Polytechnic Institute and State University, we have frequently observed flatworms consuming juvenile mussels and regurgitating empty valves. Within this facility, predation of flatworms on newly metamorphosed juveniles has at times been devastating to the propagation program, so preventing contamination of culture systems by these flatworms is a high priority. In an investigation of freshwater mussel endobionts by Curran and Benz (1996), no turbellarians were identified as living within sampled mussels, so host fish are the most probable vector for the introduction of flatworms. Therefore, to prevent the infestation of predacious flatworms, we developed guidelines for treating host fish before and during their exposure to freshwater mussel glochidia.

In this paper, we focused on flatworms of the genus *Macrostomum* (order Macrostomida, phylum Platyhelminthes), a large and common taxon found throughout the world and including 70 described species (Ferguson 1954). Species are primarily differentiated on the basis of the male penis stylet and associated glands, sperm morphology, nature of the ovary, and shape and arrangement of adenoid structures of the posterior margin. Flatworms in this genus rarely exceed 3.5 mm in length. Propulsion is by cilia on the epidermis, but they are able to adhere to the substrate by means of glandular secretions, particularly along the posterior margin. The worms possess a large mouth, a muscular pharynx, and two anterior eyespots (Figure 1).

Freshwater mussel propagation at the Aquaculture Center began in 1997. However, after 450 hatchery-reared largemouth bass *Micropterus salmoides* were introduced from a nearby private hatchery in the fall of 1999, flatworms were observed for the first time in the rearing facility. The

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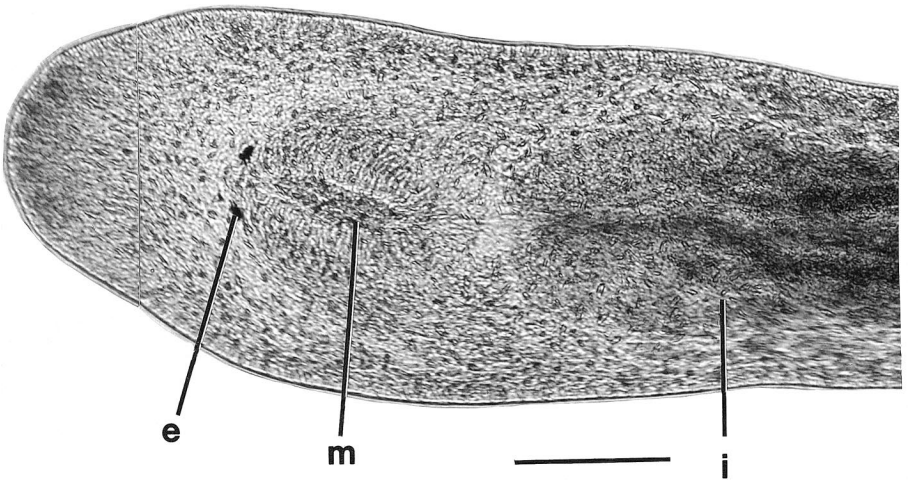

FIGURE 1.—Live *Macrostomum gigas* showing anatomical features: e = eyespot, i = intestine, and m = mouth. Scale equals 0.5 mm.

private hatchery raises a variety of freshwater fish and plants, including native and exotic species. The fish were placed in three 378-L Rubbermaid tanks that had previously been drained and bleached and filled with town water dechlorinated with sodium thiosulfate. Because no substratum, cover, or rocks were ever placed in the tanks, we believe that fish were the most likely vector for the flatworm introduction. Because the three tanks were set up in a water recirculating series, water was shared among all the tanks during the time the fish were in captivity. That is, water was circulated by a centrifugal pump through a series of ultraviolet, sediment, and trickle filters to each of the tanks and returned as overflow from standpipes.

Although *Macrostomum* sp. have been observed at several fish hatcheries used to rear freshwater mussels (L. Zimmerman, personal observations), juvenile mussel predation by flatworms is not as great in fish hatcheries with greater prey diversity as in facilities used for intensive laboratory culture.

Methods

Flatworm preparation.—Flatworms *Macrostomum* sp. were examined and identified alive using a Wild M5 dissecting microscope. A few were squash-mounted on slides to examine reproductive features. Remaining specimens were anesthetized in increasing strengths of ethyl alcohol (5% increments) and fixed in FAA (formalin, acetic acid, ethyl alcohol). Four flatworms were embedded in Paraplast, sectioned at 7-μm intervals, stained with Ehrlich's hematoxylin and Putt's eosin, cleared, and mounted in Permount. Six specimens were stained whole with 5% acid fuchsin or Erlich's hematoxylin, cleared, and mounted in Permount. All slides were placed in the Museum of Zoology, University of Massachusetts, Amherst, Massachusetts.

Predation study.—Although newly metamorphosed juvenile mussels have been observed in the guts of flatworms, a predation study was conducted to verify juvenile mortality through flatworm predation. Using a Pasteur pipette, we placed five newly metamorphosed juveniles of pheasantshells *Actinonaias pectorosa* and five flatworms into water in each 7.5-mL well of two 6-well polystyrene tissue-culture plates. At 1, 3, 6, and 24 h, we used a dissecting microscope to enumerate and examine viability of mussels and flatworms.

Toxicity tests.—Formalin was selected for toxicity testing because it is inexpensive, readily available at most aquaculture facilities, relatively safe for fish, effective against a variety of parasites and trematodes, easy to administer in large fish holding systems, and is approved by the FDA for fish disease control (Post 1987; Plumb 1999). A preliminary study was conducted to record, in stages leading to death, the behavior of flatworms exposed to 500 mg/L of formalin. Many of the flatworms reacted by contracting into a ball shape. Flatworms recovered if they were withdrawn from the formalin solution as soon as they began to constrict and placed in freshwater. We determined that the cessation of flatworm movement was not a good indicator of mortality. However, we found

FIGURE 2.—The penis stylet of *M. gigas*. Scale equals 50 μm.

that degradation of the flatworm tissue began soon after death; therefore, signs of tissue degradation were used to confirm mortality in the subsequent toxicity tests.

Four replicates of five concentrations of formalin were tested. For the treatment of ichthyobodiasis, trematodes, and ichthyophthiriasis, a shock-treatment concentration of 250 mg/L for 1 h is recommended or, alternatively, an extended exposure of 20 mg/L for 3–5 d (Post 1987). These levels are known to be safe for fish and were used as a guideline for the flatworm toxicity tests. Five exposure concentrations were tested: the recommended safe level (250 mg/L) and 25, 50, 200, and 400% of the recommended concentration (i.e., 62.5, 125, 500, and 1,000 mg/L formalin, respectively, for the shock group and 5, 10, 20, 40, and 80 mg/L for the extended group). For both the shock and extended treatments, flatworms were exposed to formalin solutions in 6-well polystyrene tissue culture plates. Flatworms were observed at 0.5, 1, 2, and 3 h for the shock group and at 1, 3, 6, 24, 48, 72, 96, and 120 h for the extended group.

Formalin concentrations were replicated four times and randomly assigned to a well in one of the four plates. Five flatworms were added to each of the wells containing 7.5 mL of formalin solution. Dishes were held at 21°C for the duration of the testing. At each of the designated sampling intervals, flatworm behavior (swimming, spastic movement, or constriction) or death (degradation) in each treatment well was noted. When not being sampled, the plates were covered to reduce evaporative losses or vaporization of the formalin. Solutions were not changed or added to during the observation period.

Juvenile toxicity tests.—Following the development of treatment recommendations for eradicating flatworms from a water system, the recommended doses were tested for their effects on newly metamorphosed juvenile mussels. Newly metamorphosed mussels were tested in a similar manner to the flatworms. Five 1-d-old wavy-rayed lampmussels *Lampsilis fasciola* were transferred with a wide-bore Pasteur pipette and randomly assigned to 1 of the 16 wells in the polystyrene tissue culture plates. Four replicates of the shock treatment (250 mg/L for 1 h), extended exposure (20 mg/L for 5 d), and controls (freshwater only) were tested. Before treatment, all juveniles had been actively feeding. Because mussel valves on newly metamorphosed juveniles are transparent, it is possible to view the guts of the mussel and observe filtering activity, which we did with a dissecting scope under 40× magnification. Mortality was defined as the cessation of filtering and pedal activity and evidence of gut tissue degradation.

TABLE 1.—Flatworm mortality from shock treatment with formalin at five concentrations. Values are the numbers surviving the treatments. Post (1987) recommended a 1-h exposure to 250 mg/L.

	Concentration (mg/L)				
Time (h)	62.5	125	250	500	1,000
0	20	20	20	20	20
0.5	20	20	20	0	0
1	20	18	0	0	0
2	18	0	0	0	0
3	0	0	0	0	0

Results

Flatworm Identification

The flatworm species in our experiment was either *Macrostomum tuba* or *Macrostomum gigas*, depending upon authority (Hyman 1943, 1951; Ferguson 1954). Specimens removed from the culture system had a long, narrow penis stylet (Figure 2) that was slightly curved and terminated in a swollen tip. The swelling is caused by a thickening of the stylet wall. It is this character that identifies the worm as *M. gigas* rather than *M. tuba*, although the two forms are very similar and may in fact be the same species. Both species are native to North America.

Predation study.—Juvenile predation and flatworm reproduction (division) were observed during the 24 h of observation. At the end of the 24-h period, only 7 of the initial 60 (11.7%) pheasantshells remained alive. Empty valves accounted for the remaining 53 pheasantshells. A total of 21 flatworms (35%) were observed with mussel shells in their guts.

Toxicity tests.—At 0.5 h, all of the flatworms in the 250-, 500-, and 1,000-mg/L treatments were dead; at 1 h, all in the 125-mg/L treatment were dead; and at 3 h, all in the 62.5-mg/L treatment were dead (Table 1). The extended (3–5 d) trial test revealed that 20, 40, and 80 mg/L formalin were sufficient to kill all flatworms within 24 h

TABLE 2.—Flatworm mortality from up to a 5-d continuous exposure to formalin at five concentrations. Values are the numbers surviving the treatments. Post (1987) recommended a 72-h exposure to 20 mg/L.

	Concentration (mg/L)				
Time (h)	5	10	20	40	80
0	20	20	20	20	20
3	20	20	20	11	0
6	20	20	20	0	0
24	20	20	0	0	0
48	20	20	0	0	0
72	20	20	0	0	0
96	20	20	0	0	0
120	20	20	0	0	0

(Table 2). Concentrations of 5 and 10 mg/L were insufficient to kill the flatworms even after 5 d.

Juvenile mussel toxicity tests.—Results from both the shock treatment (1 h at 250 mg/L) and the 3–5-d extended treatment (20 mg/L) indicate that formalin treatments on newly metamorphosed mussels were lethal because no pedal activity was observed in any of the juvenile mussels and gut tissue degradation was observed after 3 d in all treatments.

Discussion

As with all free-living platyhelminth worms, *Macrostomum* species are predacious. The mouth and pharynx have great powers of distention, and the flatworm will attempt to consume anything it contacts. Aquatic oligochaetes of the genus *Dero*, many times the size of *Macrostomum*, have been attacked and had large pieces of their body removed by *Macrostomum* flatworms (Hyman 1943).

Mollusk predation by flatworms is not isolated to our laboratory. Sickel (1998) observed the feeding by *Macrostomum* on juvenile Asian clams *Corbicula fluminea* (Müller 1774) in a laboratory. Eklu-Natey et al. (1984) reported the use of *Macrostomum* sp. for controlling snail populations (through predation) in the laboratory. The diversity of prey species in the wild probably limits the severity of predation; however, in the monoculture laboratory environment, *Macrostomum* can reproduce quickly due to the hermaphroditic nature of the worm and the abundant, easily captured prey.

Results from these toxicity tests were applied to the water recirculating systems at the Aquaculture Center. A one-time, 3-d treatment of 20 mg/L of formalin was administered to the 1,890-L host-fish holding system. During treatment, there were no fish mortalities, and immediately following the treatment, it appeared that the flatworms had been eradicated. However, after 1 week, the flatworms reappeared, presumably hatching from eggs that may be more resistant to treatment. After the reinfestation of flatworms, the system was treated again using a 3-d treatment of 20 mg/L formalin, then a 3-d waiting period, followed by a second 3-d treatment of 20 mg/L formalin. This repeated treatment seemingly eliminated the flatworms from the system. Further investigation is needed, however, to determine the life cycle of *M. gigas*, so that we can more efficiently treat the egg stage, and thereby prevent the recurrence of a flatworm epidemic.

Glochidia, once encysted on the fish (encapsulation is usually complete within 2–6 h of infestation), are reported to be extremely resistant to external toxicants (Jacobson et al. 1997). Howard and Anson (1923) and Jacobson et al. (1997) examined mortality of encapsulated glochidia exposed to various concentrations of copper solutions and found no significant difference in mortality between control glochidia and those exposed to all tested concentrations of copper. Bruno et al. (1988) found that numbers of glochidia of eastern pearlshells *Margaritifera margaritifera* encysted on Atlantic salmon *Salmo salar* were not affected by treatments of salt (NaCl in concentrations ranging from 5.4 to 33.3%), $CuSO_4$ (at 0.5 mg/L), or Nuvan (5 mg/L) followed by 1 mg/L Roccal. Given these findings and the high predation rate of flatworms on juvenile freshwater mussels in a culture setting, we recommend that systems be treated to eradicate flatworms in tanks housing infested fish before excystment.

Based on our toxicity tests, the available literature on chemical resistance of encysted glochidia, and the devastating effect of predation of flatworms on newly metamorphosed freshwater mussels, we recommend that all fish purchased from hatcheries and intended for use as hosts to transform glochidia be quarantined and treated prophylactically against predacious flatworms. Formalin treatments should be administered at concentrations of either 250 mg/L for 1 h or 20 mg/L for 5 d. Following a 3-d waiting period, the treatment should be repeated to kill newly hatched flatworms. Precautions also should be taken when introducing substrate to laboratory culture systems. Autoclaving or boiling sediments before their use for mussel culture is recommended for preventing flatworm infestations.

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