

**Report on:**  
**Observations of several mussel species made during the 3-Trifluoromethyl-4-Nitrophenol (TFM) treatment of Lewis Creek, Vermont, October 22, 2002**

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**Introduction**

Five Vermont State listed Threatened & Endangered (VT T&E) mussel species inhabit the lower three-mile reach of Lewis Creek. Bioassay derived No Observable Effect Concentration (NOEC) values for these species for TFM are 1.5-1.6 x MLC (VT F&W data). The MLC, or Minimum Lethal Concentration, is the concentration of TFM that produces 99.9 % mortality of sea lamprey ammocoetes in a 9 hr bioassay. This information implies that the targeted treatment concentration of 1.1 x MLC should not acutely effect adult individuals of these listed species. The conservative MLC target concentration was permitted because of:

- concern for other life stages of the mussels that have not been tested with TFM bioassays
- the relatively difficult nature of target concentration estimation of a permitted MLC due to highly variable pH levels in the lower Lewis Creek
- the somewhat unpredictable length of treatment duration in lower Lewis Creek due to the discharge rate, and the lake's influence on the discharge rate of the creek.

Mussels, especially juveniles are difficult to observe during treatments because flows are generally moderate to high, water temperature is cold and the mussels are often located just under or even with the bottom substrate. To allow biologists to observe them both juvenile (estimated at 3-4-year olds) and adult (estimated at 6-20 year olds) mussels of four species were collected in good numbers and placed in cages during the 2002 TFM treatment of Lewis Creek: *Lampsilis ovata* (pocketbook) adults and juveniles, *Lampsilis radiata* (eastern lampmussel) juveniles, *Lasmigona costata* (fluted-shell) adults, *Elliptio complanata*, (eastern elliptio) juveniles. In addition a limited number of five additional species were also collected and observed: *Lasmigona compressa* (creek heelsplitter), *Leptodea fragilis* (fragile papershell), *Anodontoidea ferussacianus* (cylindrical papershell), *Alasmidonta undulata* (triangle floater), *Strophitus undulatus* (creeper).

**Methods**

Mussels were collected from three rivers, the Missisquoi, Winooski, and Poultney prior to the treatment. Mussels from each river were held separately in 0.6 x 0.6 x 0.9 m wooden and, hardware cloth cages. They were transported to Lewis Creek two weeks prior to treatment on October 7 to allow them to acclimate. On October 21 the day before the 2002 treatment, the

mussels were distributed to four locations representing a control and three different anticipated factors of MLC and treatment duration in Lewis Creek Table 1. The three anticipated factors were **1**: 1.3 x MLC with a 12hr treatment from primary application point at Scott Pond Dam to below Rt 7 Bridge, **2**: 1.1 x MLC with a 12 hr treatment from above Greenbush road to about the RR crossing, and **3**: 1.1 x MLC with a 12+hr treatment from below RR crossing to the delta.

**Table 1:** Four locations representing a control and three different anticipated factors of MLC and treatment duration in Lewis Creek

	Anticipated Factors	Location
1	Control	Immediately above Scott Pond Dam, and TFM application point.
2	1.3 x MLC, 12hr	Above Quinlan Covered bridge about 300 meters.
3	1.1 x MLC, 12hr	Below Greenbush road booster point about 400 meters.
4	1.1 x MLC, 12+ hr	At the F&W boat access area.

Table 2 lists the species of mussel, life stage, and number of individuals placed in each cage at the four locations. All mussels were checked, and found to be alive and responsive prior to treatment initiation. The mussels and cages were left in place in Lewis Creek throughout the entire period of observation which lasted 7 days after the initiation of treatment.

**Table 2:** The species of mussel, life stage, and number of individuals placed in cages at four locations in Lewis Creek.

<b>Location/ Species</b>	<i>Lampsilis ovata</i> (adult)	<i>Lampsilis ovata</i> (juvenile)	<i>Lasmigona costata</i> (adult)	<i>Lampsilis radiata</i> (juvenile)	<i>Elliptio complanata</i> (juvenile)	<b>Other</b>
<b>1-Scott Pond</b>	6	6	6	6	4	
<b>2-Quinlan Covered Bridge</b>	6	8*	8	7		3- <i>Lasmigona compressa</i> -A 2- <i>Leptodea fragilis</i> -J 3- <i>Anodontoides ferussacianus</i> -A 1- <i>Alasmidonta undulata</i> -A 2- <i>Strophitus undulatus</i> -A
<b>3-Greenbush Rd</b>	6	6	7	6	6	
<b>4-F&amp;W Access</b>	6	3	6	8		

- \*Site of observed stress on one individual for 2hr period after treatment
- A= Adult mussel, J= Juvenile mussel

## Results

### **Scott Pond**

The mussels at the Scott Pond control site showed no signs of stress, and were observed filtering and responsive throughout the entire week observation period. They were observed five times the first day of treatment, and twice the day after October 23. On October 28 they were observed one last time and returned to their respective rivers of origin.

### **Quinlan Covered Bridge Site**

The TFM treatment began at the primary application site at 0730 October 22, 2002 and ended at 1935; resulting in an 12 hr treatment for the Quinlan Covered Bridge site. The measured TFM concentration at the site averaged 4.2mg/l (Chipman,B.D. 2003) during this time period. This concentration is just below the permitted 1.3 x MLC concentration, or 4.3mg/l TFM.

Observations during treatment began at 0900, at which time one juvenile *L.ovata* showed signs of narcosis with a splayed foot and slow responsiveness. All other mussels observed were filtering and responsive. During the 1100 cage observation the same *L.ovata* juvenile appeared to have

recovered, with all mussels observed filtering and responsive. The mussels were checked eight times during the first 24 hours, and except for the temporary occurrence of one stressed individual, all mussels were filtering and responsive. The mussels were then checked twice per day thru October 24 with all mussels observed to be filtering and responsive. On October 28, one week after treatment, the mussels were all observed to be filtering and responsive and were returned to their respective rivers of origin.

### **Greenbush Road**

The permitted concentration was 1.1 x MLC bioassay, pH adjusted at site, ANCP permit condition number 6. The treatment block reached the Greenbush cages at about 2030 on October 22. The first stressed Sea Lamprey “swimmers” were observed at the boost site at 2225. The mussels were checked five times prior to the block reaching the site. All mussels were observed to be filtering and responsive during these checks. At 1450 the temperature was measured at 6.5 °C, D.O. 13.2 mg/l (104% saturation), and pH 8.14. After the TFM block reached the site the mussels were checked ten times over the next 24 hour period. At all times the mussels were filtering and responsive. The mean TFM concentration was determined to be post treatment 1.6 x MLC for 11.5 hours, with a single maximum equivalent to 1.8 x MLC measured at 2330 (Chipman 2003). The permitted concentration was 1.1 x MLC bioassay adjusted by on-site pre-treatment pH data, ANCP permit condition number 6. A dead lamprey was found in the cage at 0114 on October 23. Temperature, D.O. and pH were measured at 1000 and 1345 on October 23. The measures were 5.17° C and 5.8° C, 12.24 mg/l (92.1% saturation), and 13.15 mg/l (101% saturation) D.O., and 7.89, and 7.92 pH respectively. The mussels were checked once on October 24, and again on October 28, and were found to be filtering and responsive. On October 28 they were returned to their rivers of origin.

### **F & W Access**

The permitted concentration was 1.1 x MLC bioassay, pH - adjusted, ANCP permit condition number 6. The F&W access site mussels were observed five times prior to the treatment block on Oct 22 from 11:30 to 21:38. All mussels were observed to be filtering and responsive. The treatment was first detected at 2300 on October 22. The TFM block reached the permitted concentrations of 1.1 x MLC by 0600 on October 23, and rose unanticipated to a maximum 4.3mg/l or 2.3 x MLC by 1000. For a 9hr period the measured mean concentration was 4.06mg/l TFM resulting in an adjusted MLC of 2.1. The concentration measured at the site was at or exceeded the permitted 1.1 x MLC for about 13 hrs. By 2200 on October 23 the TFM block was past the F&W access site. On Oct 23 the mussels were observed seven times from 0101 to 1400. All mussels were observed filtering and responsive. Temperature, D.O and pH were measured

three times on Oct 23 at 0930, 1115, and 1400. The temperature was 5.5<sup>0</sup> C, 5.45<sup>0</sup> C, and 6.11<sup>0</sup> C respectively. The D.O. measured 11.3mg/l (87 % saturation), 11.16mg/l (88.1 % saturation), and 10.15 mg/l (79 % saturation), and the pH 7.93, 7.81, and 7.65 respectively. On October 24 the mussels were observed twice at 1000 and 1400, and found to be filtering and responsive. The mussels were observed once more on Oct 28, found to be filtering and responsive and returned to their rivers of origin.

### **Additional Observations**

Additional in-stream resident mussel observations were made on October 23 in two reaches of lower Lewis Creek. At 1015 Lewis Creek was walked from the Greenbush cage site up about 200m. This is a section of river where long-term monitoring of an *L. ovata* population has occurred for three years to date. A total of 12 *L. ovata*, 4 *L. radiata* and 2 *E. complanata* were observed live and filtering. A number of the large Viviparid snail *Campeloma descisum* were also observed alive in this reach. At 1220 another 300m of stream located at the mid point between Old Rt. 7 bridge and Greenbush Road was walked. This is the second long-term *L. ovata* monitoring reach. A total of four *L. ovata* and four *L. costata* were observed alive and filtering in this reach. The Vermont F&W Dept personnel did non-target searches by stream section for the entire length of treated stream. During their searches no recently killed mussels were observed, with very few mussels actually observed. One *L. costata* however was found in the section of river surveyed above Ferrisburg Falls, (the first boost site) and verified by Mark Ferguson. This observation is the first record of *L. costata* above the Ferrisburg Falls.

### **Summary and Discussion**

**All the caged mussels observed during the treatment survived**, and with the exception of one juvenile showed no signs of stress. A single *Lampsilis ovata* juvenile was observed with a splayed (extended) foot and responded slowly to stimulus during the first two hours of exposure, and then recovered. The stressed *L. ovata* juvenile was caged below the primary application point at the Quinlan Covered Bridge site. All mussels were left in place for six days post treatment with no mortalities observed, at which time they were returned to their respective rivers.

The toxicity of TFM increases as pH decreases. Monitoring of TFM concentrations, and pH in the lower Lewis Creek during treatment, and subsequent data analysis by VT F&W showed that actual TFM toxicity at the mussel cage sites were 1.3 x MLC for 12 hrs Quinlan Covered Bridge; 1.6 x MLC for approximately 10 hrs below Greenbush Road; and 2.1 x MLC for approximately 9 hrs, and maintained above 1.1 x MLC for 13 hrs at the F&W boat launch (Chipman,B.D. 2003)

The primary reasons for the high estimated toxicity at the lower sites is the unexpected decrease in pH from one day to the next during treatment, and the resulting overestimation of the target concentration of TFM mg/l needed to achieve the permitted 1.1 x MLC in the lower river. Fortunately, continuous monitoring of stream pH by the treatment team detected the dropping pH levels, and terminated the lower Greenbush road boost. Despite the unexpected and uncontrollable fluctuations in stream chemistry during treatment and resulting high estimated toxicity in the Lower Lewis Creek none of the caged mussels appeared stressed or died; in addition no dead mussels were found during follow up stream walks.

Based on the available bioassay information one would have expected a few mussels to show signs of stress, and possible mortality. During the 1990 treatment *Lampsilis radiata* adults showed signs of stress when exposed to TFM concentrations estimated at 1.0-1.2 x MLC for 16hrs (Fiske memo 1991). One possible explanation is the considerably colder water temperature during the 2002 treatment 5-7 °C, compared to the 1990 treatment 14-16 °C (Anderson, J.K 1990). The lower temperatures may result in a reduced uptake of the chemical by the mussels due to slower metabolic rates associated with the cold temperature. Most of the bioassay derived NOEC data for the mussels was at higher temperatures of 15-20 °C. If colder temperatures during the treatment prevented the metabolic uptake of chemical by the mussels it does not appear to have had the same effect on the Sea Lamprey ammocoetes, as 100% of caged ammocoetes were killed (Chipman, B.D. 2003).

Another possible contributing factor is the length of exposure time between the two treatments. At the F&W access site in lower Lewis Creek the 1990 treatment lasted 16hrs (Anderson, J.K 1990). During the 2002 treatment TFM concentrations dropped off rapidly after 9hrs at 2.1 x MLC, with the 1.1 x MLC target lasting about 13 hrs. Several studies have shown that the length of exposure time can increase the percent mortality or non-lethal effects on mussels (Bills 1992, Maki 1975).

The caged mussel observations and the post treatment in-stream observations increase the confidence that the 2002 Lewis Creek treatment did not adversely affect the juvenile and adult Threatened and Endangered listed mussels, from acute lethal effects. The estimated toxicity of TFM during the 2002 Lewis Creek treatment determined from water testing during the treatment was significantly higher (1.6 and 2.1 x MLC) than the 1.1 x MLC permitted for the lower Lewis Creek by the VTDEC, ANCP. The higher predicted toxicity values were determined from in-stream measures of pH and TFM concentration,

and were due to an unanticipated drop in stream pH during the overnight hours of the 2002 treatment. Based on bioassay data, and observations of non-target impacts during the 1990 treatment, a predicted 1.6 to 2.1 x MLC should have resulted in higher observable non-target impacts in lower Lewis Creek. It appears however that lower stream temperatures, and possibly shorter exposure periods compared to the 1990 treatment were likely responsible for the no observable impact on the caged adult and juvenile mussels, and other non-target species during the 2002 treatment. In conclusion the permitted 1.1 x MLC was not conservative enough to prevent the actual treatment toxicity from uncontrollably increasing to levels that potentially placed the Threatened and Endangered mussel populations in Lewis Creek at a significantly greater risk to stress and some mortality than is desirable.

Based on the information gathered during this treatment it is recommended:

- That future treatments of Lewis Creek be permitted at a lower concentration relative to MLC, and or that the treatments be conditioned to occur at temperatures below 10° C.
- The use of caged mussel monitoring during treatments to allow for a better evaluation of the potential effects of each treatment on the native mussel populations of Lewis Creek.
- Adequate monitoring of stream pH, TFM conc., Temperature, and exposure period be a part of all future treatments.
- The relationship of an MLC- based concentration to predict the toxicity of TFM to non-target species of concern should continue to be studied to better understand how other environmental factors such as pH, temperature, and exposure period may influence it.

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