GREAT LAKES FISHERY COMMISSION

Project Completion Report[1]

Distribution of Niclosamide Following Granular Bayluscide Applications in Lotic Systems

by:

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ABSTRACT

The granular formulation of Bayluscide [Bayluscide 3.2% Granular Sea Lamprey Larvicide, granular Bayluscide (gB)] is applied in lentic and lotic systems to survey (assessment) and kill (treatment) larval sea lampreys (*Petromyzon marinus*; Linnaeus, 1758) in the Great Lakes basin. Granules are spread on the water surface, settle to the sediment surface, and dissolve. The potential risk of niclosamide exposure (2′,5-dichloro-4′-nitrosalicylanilide), the active ingredient of gB, to non-target organisms located downstream of survey plots, is a concern of partner agencies (state-level natural resource departments, U.S. Fish and Wildlife Service Ecological Services, and Fisheries and Oceans Canada Species at Risk Branch). Spatiotemporal distribution of niclosamide in the water column and sediment was evaluated in and downstream of five larval survey plots in two rivers following the application of gB. Water samples were collected at 0.25, 2, 4, 6, 8, and 24 h from three depths in the water column (10 cm above the sediment, ½ the water column depth, and the water surface) at three locations inside each survey plot, and 1 meter upstream from three sediment sample grids positioned 10, 30, and 100 m downstream. Sediment samples were collected from inside the grids at 0.25, 2, 4, 6, 8, and 24 h, and from inside the survey plots, 8 and 24 h after gB application. Niclosamide was detected in the sediment and water at all sample locations. From 2 to 24 h after application, average water concentrations 1) varied between study sites, 2) decreased from the survey plots to 100 m downstream, 3) varied by depth in the water column, and 4) decreased over time. Average sediment concentrations varied by distance downstream and time post-application, but not by study site or river. Data suggest there would be negligible exposure to non-target organisms downstream of a gB survey plot based on low niclosamide concentrations measured in the water and sediment. The depletion rate of niclosamide was also evaluated

in St. Clair River sediment dosed at the field application rate. Niclosamide concentration decreased at a rate of 2.28% per hour over the 24 hours measured, equating to a half-life of 1.27 days. This indicates the length of time an organism in the sediment in a survey plot might be exposed. Underwater cameras were placed along the edge of two St. Clair River survey plots to document gB distribution on the sediment and any potential target and non-target effects. Video was inconclusive in tracking gB through the water column. Larval sea lamprey and non-target mortality were not observed. Additional video footage of one St. Clair River survey plot showed large areas of river bottom without gB.

INTRODUCTION

Sea lampreys (*Petromyzon marinus*; Linnaeus, 1758) are controlled in the Great Lakes basin using the lampricides TFM (3-trifluoromethyl-4-nitrophenol) and niclosamide (2′,5-dichloro-4′-nitrosalicylanilide). Bayluscide is the aminoethanol form of niclosamide. The granular formulation of Bayluscide [Bayluscide 3.2% Granular Sea Lamprey Larvicide, granular Bayluscide (gB)] is applied as a control tool in lentic and lotic systems too large to be treated economically with liquid lampricide formulations, and as a survey tool to assess for larval sea lamprey presence and abundance in water too deep for backpack electrofisher gear. Granular Bayluscide is applied to the water surface at a rate of 17.5 $g \cdot m^{-2}$ with customized equipment and boats, irrespective of water pH, alkalinity, depth, and flow rate (Barber and Steeves, 2021). Granules sink to the sediment surface where the active ingredient (niclosamide) is released. The potential of niclosamide exposure to non-target organisms located downstream of survey plots has been a concern of partner agencies (state-level natural resource departments, U.S. Fish and Wildlife Service Ecological Services, and Fisheries and Oceans Canada Species at Risk Branch). Exposure of freshwater mussels is of particular concern because their mobility is limited and niclosamide, used to eliminate snails, has proven toxic to other molluscs (Kilgour and Baker, 1994; Dawson, 2003; McDonald and Kolar, 2007; Costa et al., 2008; Dai et al., 2008; Luoma et al., 2018;

Barbour et al., 2021). Niclosamide residues in water and sediment in standard-size survey plots (25 × 20 m) and downstream reaches following gB application in lotic environments has not been evaluated. Knowledge of niclosamide concentrations in water and sediment is necessary to estimate exposure and assess risk to non-target organisms, and is the primary focus of this study. This work addresses the Sea Lamprey Control Board's research priority theme number 6: w*hat are the physiological effects of lampricide treatments on non-target organisms, particularly species of concern including, but not limited to, lake sturgeon, mudpuppies, native lamprey, native mussels and their hosts* (GLFC, 2022).

OBJECTIVES

1) Evaluate the spatiotemporal distribution of niclosamide in the water column and sediment, in larval survey plots and sediment sample grids located 10, 30, and 100 m downstream, in two lotic systems.
    - This work was conducted at two sites in the Au Train River (July 8 – 11, 2015: Alger County, Michigan) and at three sites in the St. Clair River (June 3 – 8, 2016: St. Clair County, Michigan).
        - ~ Low concentrations of niclosamide were detected in the water column at all depths in all survey plots and grids.
        - ~ Over time, average niclosamide concentrations in survey plot and grid water decreased but was detected throughout the 24 h sample period.
        - ~ Niclosamide concentrations were typically greatest in the survey plots at 10 cm above the sediment surface during the 0.25 h sample period.
        - ~ Niclosamide was detected in the sediment in all survey plots and grids.
        - ~ Niclosamide concentrations in survey plot sediment at 8 and 24 h were greater than in the three grids during all sample periods.

- ~ Between the grids, average niclosamide concentrations in the sediment were greatest at 10 m and decreased downstream.
- ~ Partitioning of the chemical from the water into the sediment, as it was carried downstream by the flowing river water, is the probable source of niclosamide at 30 and 100 m, rather than the deposition of gB. There is a chance that some gB deposited into the 10-m grid.

2) Determine the depletion rate of niclosamide in sediment dosed with gB in the St. Clair River (June 2 – 3, 2016).
   - Niclosamide concentrations in St. Clair River sediment, dosed at the standard field application rate, were sampled over a 24 h period (June .
   - The dissipation rate of niclosamide from sediment was calculated to be about 2.28% per hour which corresponds to a half-life of 1.27 days.
3) Record video footage of survey plots with underwater cameras to document gB distribution on the sediment, larval sea lamprey mortality, and the potential effects of niclosamide to non-target species (June 3 – 6, 2016).
   - This work was conducted at two St. Clair River sites.
     - ~ Technical difficulties with the camera system resulted in little usable footage.
     - ~ The silty stream substrate reflected light back to the camera and blended with the green water.
     - ~ Additional video footage taken with a GoPro® of one survey plot showed large areas (not quantified) of river bottom void of granules.

METHODS - OBJECTIVE 1

Spatiotemporal Distribution of Niclosamide in Water and Sediment

Study Site Locations, Setup, and Sample Collection

Tests were conducted at two sites in the Au Train River [Au Train 1 and 2; Lake Superior, Alger County, Michigan (Figure 1, Table 1)] and at three sites in the Middle Channel of the St. Clair River [St. Clair 1–3; Lake St. Clair, St. Clair County, Michigan (Figure 2, Table 1)]. Each site included a standard-size survey plot (25 m long upstream/downstream × 20 m wide) that Sea Lamprey Control Program (SLCP) uses to assess for larval sea lamprey, and sample sediment grids (6 m long upstream/downstream × 18 m wide) placed 10, 30, and 100 m downstream (Figures 3, 4, and 5). Each grid was divided into 18 sample sections (3 m long × 2 m wide upstream/downstream). Water samplers (Figure 6) were deployed in the center of each survey plot and 1 m upstream from each grid, at ¼, ½, and ¾ the width (Figure 3, 4, and 5). Each sampler was designed to draw water from 8 ports spaced equally across a 1-m horizontal span at 10 cm above the sediment and ½ the water column depth (½ the WC; 50–80 cm). Each water line was 3.63–3.69 m long (1.6 mm inside diameter), made of polytetrafluoro-ethylene, and had a total volume of 7.2–7.3 mL. Each plot was setup and control samples collected the day prior to gB application.

Granular Bayluscide Application and Sample Timing

Granular Bayluscide was applied to each survey plot at a rate of 8.74 kg·500 m^{-2} (Barber and Steeves, 2021) with a motorized seed spreader mounted to the front of a boat. The boat made several passes (4–6 in about 15 minutes) through the survey plot to achieve an even distribution of gB. Time zero commenced when the application was completed. Water and sediment samples were collected 0.25, 2, 4, 6, 8, and 24 h after application. During each sample period, a person in a kayak collected water from the three samplers and measured pH (Thermo Scientific, Orion Star A326) and temperature at 0.5 m (± 0.1 m) below the water surface in the survey plot. Water and sediment samples were collected from the grids with boats propelled by oars (Au Train River) or a 10-horsepower motor (St. Clair River). Motors were turned off prior to entering the study site and the boats pulled into position to avoid disturbance of the water column and granules on the sediment surface. Samples from grids and associated water samplers were collected from downstream to upstream. Each boat was navigated to one of the three water samplers (Figure 6) located immediately upstream of a grid and samples were collected in unison. Sediment was then sampled in the three pre-designated grid sections (Figure 4). Sediment was also collected in the survey plot at 8 h (between the downstream edge and 6.25 m upstream) and at 24 h (between 6.25 m and 12.5 m upstream of the downstream edge; Figure 3).

Water Samples

Prior to collection from 10 cm above the sediment and ½ the WC depth (Figure 6), approximately 25 mL of water were drawn with a 30-mL syringe (Becton Dickson, Franklin Lakes, New Jersey) to purge the lines of the previous sample. All control and test water samples were collected in the following order: surface (2 cm below the surface), 10 cm above the sediment, and ½ the WC depth. All water was collected with a 3-mL syringe (Becton Dickson, Franklin Lakes, New Jersey) and duplicate 1-mL aliquots were transferred to two pre-labeled 2-mL cryogenic microcentrifuge vials (USA Scientific, Ocala, FL). Water sample data collected for this study are available in Kaye et al. (2022).

Sediment Samples

During each sample period, a Petite Ponar Grab (Wildco, Yulee, Florida) was used to collect two sediment samples from the three pre-designated sections of each grid (Figure 4). The two samples from each section were placed into a pre-weighed polypropylene bag (4 mm thick, 60.96 × 91.44 cm, United States Plastic Corp, Lima Ohio). The supernatant was decanted, measured, and discarded. Each bag was then mixed by hand for one minute and weighed. Duplicate sub-samples (15–20 g each) were placed into two tared, pre-labeled 50-mL centrifuge tubes and weighed. Methanol (10 mL) was added to each centrifuge tube. The tubes were then capped and mixed by inversion. All samples were placed in a portable freezer (−20°C) in the field and then stored (water: −80°C, sediment: −20°C) at the U.S. Geological Survey Upper Midwest Environmental Sciences Center (UMESC) until they could be further processed and analyzed for niclosamide. Sediment sample data collected for this study are available in Kaye et al. (2022).

Total Organic Carbon

Two sediment grab samples (about 1,600 cm^3 each) were randomly collected downstream from each grid with a Petite Ponar Grab immediately after site setup, combined in a clean stainless-steel container, and mixed. Two 1-L sub-samples were placed into separate 1.89-L glass jars. The jars were placed in a freezer while in the field (−20°C) and then stored at UMESC (−20°C) until analyzed for total organic carbon (TOC) using American Society for Testing and Materials method D 2974-87 by Davy Laboratories (La Crosse, Wisconsin).

Sediment and Water Stability Samples

Sediment and water stability samples were collected and fortified with niclosamide in the field and laboratory. All samples were frozen in the field (–20°C) and then transported to UMESC where they were held (–80°C) until they could be further processed and analyzed for niclosamide. These samples were then used to correct for niclosamide degradation, extraction efficiency, and laboratory precision.

Field fortified sediment samples (n = 6, 15–20 g) were prepared by transferring sediment to 50-mL centrifuge tubes, adding 1 mL of 100 $\mu g \cdot mL^{-1}$ niclosamide in methanol, diluting with 9 mL methanol, capping the tubes, and mixing by inversion. Lab fortified samples (n = 6, 15–20 g) were prepared in the same manner except niclosamide was added immediately prior to analysis.

Field fortified water samples (n = 6) were prepared in 50-mL volumetric flasks by adding 1 mL of 100 µg·mL^{-1} niclosamide in methanol, filling the flask to volume with river water, and mixing. Duplicate 1-mL aliquots were transferred to 2-mL cryogenic microcentrifuge vials. Lab fortified samples (n = 6) were prepared in the same manner except niclosamide was added immediately prior to analysis.

Sample Preparation

Sediment samples were warmed to ambient temperature and niclosamide was extracted with four separate methanol elutions. For each elution, 10 mL of methanol (the first addition was in the field) was added to the 50-mL centrifuge tube. The tube was agitated for five minutes on a laboratory shaker (3589 Multi-Wrist Shaker, Lab-Line, Melrose Park, Illinois), centrifuged at 5,000 relative centrifugal force (RCF) for 10 minutes at 20°C (Avanti J-26 XPI; Beckman Instruments, Palo Alto, California). Supernatant from all four elutions were combined in a single 50-mL volumetric flask. E-pure water (20 mL; 18 MΩ/cm resistance water) was added and mixed with the supernatant by inversion, cooled to 20°C, and brought to final volume (50 mL) with methanol. Following a second inversion, a 1-mL aliquot was transferred to a 2-mL microcentrifuge tube and centrifuged at 10,000 RCF for 10 minutes at 20°C (Avanti 30; Beckman Instruments, Palo Alto, California). Approximately 500 µL of the supernatant was transferred to a liquid chromatography vial and niclosamide quantified.

Water samples were warmed to ambient room temperature, diluted 10 fold with methanol, mixed by inversion, and transferred to 2-mL centrifuge tubes. Samples were centrifuged at 15,000 RCF for 10 minutes at 20°C using a Beckman Instruments Avanti 30 centrifuge, and approximately 500 µL of supernatant was transferred to a liquid chromatography vial and niclosamide quantified.

Niclosamide Quantification

Niclosamide was quantified on a binary Agilent model 1260 liquid chromatograph (Agilent Technologies, Santa Clara, California) system equipped with a Kinetex, 2.6 µ, XB-C_{18}, 50 × 2.1 mm liquid chromatography column (Phenomenex, Torrance California) and Agilent model 6460 triple quadrupole mass detector with an Agilent Jet Stream electrospray ionization source. The injection volume for samples and standards was 5 µL. Niclosamide calibration standards (0.0027–2.0000 $\mu g \cdot mL^{-1}$) and all samples were chromatographed in mobile phase (A = 750 mL E-pure water + 250 mL mass spectrometry grade methanol + 385 mg mass spectrometry grade ammonium acetate; B = 1000 mL mass spectrometry grade methanol + 385 mg ammonium acetate) and a 0.60 $mL \cdot minute^{-1}$ flow rate. A 2.75-minute mobile phase gradient starting at 20.0% B and switching to 80.0% B at 1.50 minutes, holding 80.0% B until 2.25 minutes, and back to 20.0% B at 2.30 minutes, was used to elute niclosamide. The source operated in negative mode with the gas (350°C and 9.0 $mL \cdot minute^{-1}$), nebulizer (25 pounds per square inch), sheath gas (400°C and 11.0 $L \cdot minute^{-1}$), capillary (−3000 volts), and nozzle (−500 volts) parameters held stationary. The fragmentor voltage was set at 130 and transitions 325→289 and 325→171 were monitored. Samples that exceeded the calibration curve were diluted with methanol and re-injected. Calibration standards were considered acceptable for use only if the deviation of two independently prepared sets of standards were within 5%. Each sample set was bracketed by a full set of calibration standards. Two calibration standards were injected after every 10 samples. After every 30 samples, a full set of calibration standards was injected. Calibration curves (quadratic, origin ignored, weighting 1/x, $R^2 \geq 0.995$) were calculated (MassHunter Software, Agilent Technologies, Santa Clara, California) and used to compute sample concentrations. Maximum acceptable ion ratio variance was ± 10%.

Data Analysis

Statistical analyses were conducted using RStudio (version 3.6.1; R Core Team 2019). Simple descriptive statistics were used to determine the mean and standard deviation of niclosamide concentrations in the water and sediment. Linear mixed effects regression (LMER) analysis (Bates et al., 2015) was used to predict niclosamide concentrations (dependent variable) in the water based on site, depth in the water column, distance downstream, and time post gB application (independent variables).

An analysis of variance (ANOVA) table, generated with the LMER fitted model, was used to assess the variability of mean niclosamide concentrations in the water based on site, distance downstream, depth in the water column, and time post gB application (Bates et al., 2015; Montgomery, 2017).

LMER analysis was used to predict niclosamide concentrations (dependent variable) in the sediment based on site, distance downstream, and time post gB application (independent variables). An ANOVA table, generated with the LMER fitted model, was used to assess the variability of mean niclosamide concentrations in the sediment based on site, distance downstream, and time post gB application.

METHODS - OBJECTIVE 2

Depletion Rate of Niclosamide in St. Clair River Sediment

<u>Study Site Location, Setup, and Sample Collection</u>

Fifteen sediment sample trays were deployed upstream of a dock (Table 1) that extended 31 m out into the Middle Channel of the St. Clair River, at a depth of about 2 m. Each tray (22.9 × 33.0 cm) contained three glass jars (88.7 mL, ID 6 cm) placed in a custom cement mold cast. Jars were filled 3 mm from the top with 299–310 g of St. Clair River preferred larval sea lamprey sediment (USFWS, 2018). All jars were dosed, except for those in the control tray, with about 0.0496 g of gB, the field application rate (FAR; Barber and Steeves, 2021), based on the following equation:

$$\text{granular Bayluscide } (g) = FAR\left(\frac{g}{m^2}\right) \times \frac{1\ (m^2)}{100^2\ (cm^2)} \times \pi \times \left[\frac{\text{ID } (cm)}{2}\right]^2$$

where FAR is 17.5 g·m^{-2} and ID is the inner diameter (6 cm) of the top of the jar. Three additional jars were dosed at the same rate. Methanol (35 mL) was added to each jar which were then capped and mixed by inversion. The samples were analyzed in the laboratory to determine storage stability and extraction efficiency.

To deploy, trays were covered with weighted tops and lowered to the river bottom via fishing line (4 m long, 22.7 kg test) tied to four small eyebolts anchored in the corners of each tray. The line was threaded through the four corners of a plastic cover weighted with cement ballast. The weight served to seal the cover to the tray, prevent scour of the sediment and gB during deployment, and to reach and seal the sampler quickly during retrieval. Once trays were placed on the stream substrate, the covers were pulled to the surface via the lines. Two trays (tray = replicate) were retrieved at 0.25, 1, 2, 4, 6, 8, and 24 h starting with those closest to shoreline. The covers were lowered onto the trays, guided by the lines, and the samplers then pulled to the surface. Surface water was removed from each jar, the volume measured, mixed, and duplicate 1-mL aliquots were transferred to 2-mL centrifuge tubes. The control tray was not placed in the river. All jars (control and treatment) were capped and mixed by inversion following the addition of 35 mL of methanol.

Sample Preparation

Water samples were diluted with methanol 5-fold by weight and centrifuged at 15,000 RCF for 10 minutes at 20°C on a Beckman Instruments Avanti 30 centrifuge. About 500 µL was transferred to a liquid chromatography vial for analysis. To extract niclosamide from the sediment, 35 mL of methanol was added (the first addition was in the field) to each jar which were then mixed for 5 minutes on a laboratory shaker (High Speed, Mistral Multi-Mixer, Lab-Line Instruments, Melrose Park Illinois) and centrifuged at 500 relative centrifugal force at 20°C on a Beckman Instruments Avanti J-26 XPI centrifuge for 5 minutes. The supernatant was transferred to a common 250-mL volumetric flask. The extraction procedure was repeated three additional times for each sample jar. Each 250-mL volumetric flask with the 4 supernatants was brought to volume with methanol. A 1-mL aliquot was transferred to a 20-mL glass scintillation vial and diluted 10-fold by weight with methanol. A 1-mL aliquot of the methanol diluted sample was transferred to a 2-mL centrifuge tube and centrifuged at 15,000 RCF for 10 minutes at 20°C on a Beckman Instruments Avanti 30 centrifuge. Approximately 500 µL was transferred to a liquid chromatography vial and analyzed for niclosamide concentration using the methods described in the niclosamide quantification section.

To determine the dilution precision, 0.0496 grams of gB were transferred directly into three 250-mL volumetric flasks and brought to volume with methanol. Three 1-mL aliquots from each volumetric flask were placed in a 20-mL scintillation vial, diluted 10-fold by weight with methanol, centrifuged at 15,000 RCF for 10 minutes at 20°C on a Beckman Instruments Avanti 30 centrifuge, and then assayed for niclosamide using the methods described in the niclosamide quantification section.

METHODS - OBJECTIVE 3

Underwater Camera Footage of a Granular Bayluscide Application

Nineteen underwater video cameras were placed along three sides (downstream and the two 25 m sides) of the St. Clair 1 and St. Clair 2 survey plots to document gB distribution on the sediment, larval sea lamprey mortality, and the potential effects of niclosamide to non-target species. Starting 0.61 m upstream from the downstream edge, on each 25 m long side, a 0.61 m long piece of rebar was driven about 0.30 m into the sediment every 1.22 m. One rebar was also placed in the center of the 20 m long downstream side of the survey plot (Figure 7). A concrete block, placed on end, was secured to each rebar with a bungee cord.

Camera modules, built by the Stockbridge High School Robotics Team (Stockbridge, MI), were connected to two batteries, a voltage regulator, and a Raspberry Pi microcontroller (Raspberry Pi Foundation, Cambridgeshire, United Kingdom). The system had five megapixel resolution, a 5 hour runtime, and the capacity to record one terabyte of data. The camera setup was placed inside individual waterproof plastic tubes and secured with a bungee cord to the top of each concrete block, approximately

0.46 m above the river bottom. Each camera view was about 1.22 m wide and extended approximately 1.22 m into the survey plot. All cameras were programmed to start recording about 1 hour before the gB application.

A GoPro® camera, mounted on the end of a PVC (polyvinyl chloride) pipe, affixed to the back of a kayak, was used to record digital video of the sediment surface at one survey plot. The hands-free design allowed the kayak to be paddled across the survey plot as the GoPro® recorded.

RESULTS - OBJECTIVE 1

Spatiotemporal Distribution of Niclosamide in Water and Sediment

Niclosamide in Water

All Study Sites

The greatest niclosamide concentrations in water were typically in the survey plots at 10 cm above the sediment during the 0.25 h sample period (Table 3–5). However, at the Au Train 2 site, the average concentration in the 100-m grid was greater than in the survey plot and at 10 and 30 m for this sample period (Table 4). This could be due to the sampling of an apparent plume of niclosamide that traveled downstream from the survey plot shortly after gB was applied. This was observed at all sites and the concentration detected was likely due to the rate of niclosamide dissolution into the water, the time at which samples were collected, and stream flow at each site. The stream flow of 0.15 $m \cdot sec^{-1}$ measured on the Au Train River equates to 135 m travel in 15 minutes. At the St. Clair River sites, water from the survey plots would have traveled 114 m (St. Clair 1), 225 m (St. Clair 2), and 279 m (St. Clair 3) in 15 minutes.

Niclosamide concentrations in the water column for all study sites combined during the 0.25–24 h sample period averaged 0.36 $\mu g \cdot L^{-1}$ (+/- 1.51 SD, Range 0.00–27.87 $\mu g \cdot L^{-1}$) in the survey plots, 0.23

μg·L^{-1} (+/- 0.57 SD, Range 0.00–4.56 μg·L^{-1}) in the 10-m grids, 0.24 μg·L^{-1} (+/- 0.63 SD, Range 0.00–0.81 μg·L^{-1}) in the 30-m grids, and 0.56 μg·L^{-1} (+/- 2.06 SD, Range 0.00–16.48 μg·L^{-1}) in the 100-m grids. Including data from the 0.25 h sample period indicates that concentrations were similar between study sites ($F = 0.16_{(3, 984)}$; $P = 0.92$), did not vary by distance downstream ($F = 0.76_{(1, 984)}$; $P = 0.39$), but varied by depth in the water column ($F = 9.52_{(2, 984)}$; $P \leq 0.01$), and decreased over time ($F = 31.32_{(1, 984)}$; $P \leq 0.01$). When the 0.25 h data (the initial plume) are removed, concentrations averaged 0.11 μg·L^{-1} (+/- 0.35 SD, Range 0.00–6.53 μg·L^{-1}) in the survey plots, 0.12 μg·L^{-1} (+/- 0.22 SD, Range 0.00–1.33 μg·L^{-1}) in the 10-m grids, 0.073 μg·L^{-1} (+/- 0.10 SD, Range 0.00–0.81 μg·L^{-1}) in the 30-m grids, and 0.062 μg·L^{-1} (+/- 0.091 SD, Range 0.00–0.78 μg·L^{-1}) in the 100-m grids (Table 3, Figure 8). Water concentrations varied between study sites ($F = 10.34_{(3, 804)}$; $P \leq 0.01$), decreased from the survey plots down through the 100-m grid ($F = 8.98_{(1, 804)}$; $P \leq 0.01$), varied by depth in the water column ($F = 21.03_{(2, 804)}$; $P \leq 0.01$), and decreased over time ($F = 14.64_{(1, 804)}$; $P \leq 0.01$). The niclosamide concentration profile in each river was unique and warrants individual description.

Au Train River Study Sites

Average niclosamide concentrations in the water from 0.25–24 h at the Au Train 1 and Au Train 2 sites varied by time post-application, ($F = 224.24_{(1, 423)}$; $P \leq 0.01$), and depth in the water column ($F = 5.90_{(2, 423)}$; $P \leq 0.01$), but did not vary by distance downstream from the survey plot ($F = 1.81_{(1, 423)}$; $P = 0.18$) (Table 4, Figure 8). Again, this was due to the Au Train 2 site where six individual samples in the 100-m grid at 0.25 h had higher concentrations than the survey plot. When the 0.25 h sample data (niclosamide plume) are removed, concentrations varied by time post-application ($F = 9.64_{(1, 351)}$; $P \leq 0.01$), depth in the water column ($F = 15.02_{(2, 351)}$; $P \leq 0.01$), and distance downstream from the application ($F = 8.40_{(1, 423)}$; $P \leq 0.01$).

At the Au Train 1 site, water concentrations in the survey plot remained greater than in downstream grids at 10 cm above the sediment surface up to 8 h. Water concentrations at ½ the water column and the water surface were mostly greater in the grids than in the survey plot except for the 100-m grid after hour six (Figure 8). Au Train 2 site had a similar concentration profile during the 0.25 h sample period at ½ the water column and the water surface. However, concentrations during the same time, 10 cm above the sediment, were greatest in the 100-m grid (Figure 8). This study site is where the most notable niclosamide plume was measured during the 0.25 h sample period.

St. Clair River Study Sites

Average niclosamide concentrations in the water from 0.25–24 h at the three St. Clair River sites varied by time post-application ($F = 13.37_{(1, 560)}$; $P \leq 0.01$) and water column depth ($F = 7.08_{(2, 560)}$; $P \leq 0.01$), but did not vary by distance downstream ($F = 2.01_{(1, 560)}$; $P = 0.16$) (Table 5, Figure 8). When the 0.25 h sample data (niclosamide plume) are removed, concentrations varied by time post-application ($F = 24.66_{(1, 452)}$; $P \leq 0.01$), depth in the water column ($F = 14.82_{(2, 452)}$; $P \leq 0.01$), but still did not vary by distance downstream ($F = 1.93_{(1, 452)}$; $P = 0.17$). Niclosamide concentrations measured at 24 h were generally greater than the two Au Train River sites at all heights and decreased less over time at the St. Clair 2 and 3 sites.

<u>Niclosamide in Sediment</u>

Niclosamide was detected up to 24 h in all survey plot and grid sediment samples, except at 100 m in the Au Train River (Table 6-8, Figure 8). Niclosamide concentrations for all study sites combined varied by distance downstream ($F = 26.22_{(1, 242)}$, $P \leq 0.01$), and time post-application ($F = 9.30_{(1, 242)}$, $P \leq 0.01$), but did not vary by study site ($F = 0.75_{(3, 242)}$, $P = 0.53$), or by river ($F = 0.97_{(1, 242)}$, $P = 0.33$).

Average niclosamide concentrations in survey plots at 8 and 24 h were greater than in the grids during all sample periods (Tables 6-8, Figure 8). Niclosamide concentrations in the sediment averaged 421.85 $\mu g \cdot kg^{-1}$ (86.49–1,182.39 $\mu g \cdot kg^{-1}$) at 8 h and 312.50 $\mu g \cdot kg^{-1}$ (24.21–1,090.60 $\mu g \cdot kg^{-1}$) at 24 h for the two rivers combined. Between the grids, average sediment concentrations were greatest at 10 m and decreased downstream.

Au Train River Study Sites

Average niclosamide concentrations in the sediment from 0.25–24 h at the Au Train 1 and 2 sites varied by distance downstream from the survey plot ($F = 10.86_{(1, 104)}$; $P \leq 0.01$) but not by time post-application ($F = 3.11_{(1, 104)}$, $P = 0.08$; Table 7, Figure 8). When the 0.25 h sample data (niclosamide plume) are removed, concentrations still varied by distance downstream from the survey plot ($F = 9.55_{(1, 86)}$; $P \leq 0.01$) but not by time post-application ($F = 1.83_{(1, 86)}$; $P = 0.18$).

St. Clair River Study Sites

Average niclosamide concentrations in the sediment from 0.25–24 h at the St. Clair 1–3 sites varied by distance downstream from the survey plot ($F = 16.27_{(1, 139)}$; $P \leq 0.01$) and by time post-application [($F = 5.84_{(1, 139)}$, $P = 0.02$; Table 8, Figure 8). When the 0.25 h sample data (niclosamide plume) are removed, concentrations still varied by distance downstream from the survey plot ($F = 16.28_{(1, 112)}$; $P = 0.01$) and by time post-application ($F = 7.21_{(1, 112)}$; $P \leq 0.01$).

Water Chemistry and Total Organic Carbon

Water pH averaged 8.08 and 8.36 at Au Train 1 and 2 plots, respectively. Water pH averaged 8.15, 8.13, and 8.17 at St. Clair 1, 2, and 3 plots, respectively. Water temperature averaged 24.48 and 26.38°C at Au Train 1 and 2 plots, respectively. Water temperature averaged 14.95, 15.93, 15.53°C at St. Clair 1, 2, and 3 plots, respectively (Table 2). Total organic carbon (TOC; dry weight) averaged 6,440 $mg \cdot kg^{-1}$ (0.64%) and 13,400 $mg \cdot kg^{-1}$ (1.34%) at Au Train 1 and 2 plots, respectively. TOC averaged 8,620 $mg \cdot kg^{-1}$, (0.86%), 9,160 $mg \cdot kg^{-1}$ (0.92%), and 3,320 $mg \cdot kg^{-1}$ (0.33%) at St. Clair 1, 2, and 3 plots, respectively. Silt and clay components at the study sites were not measure. However, the sediment observed at the three study sites, and on the shallow water shelves throught the St. Clair River, was composed of silt and clay with little to no sand or course organic material.

RESULTS – OBJECTIVE 2

Depletion Rate of Niclosamide in St. Clair River Sediment

Niclosamide concentration (Table 9, Figure 9) in St. Clair River sediment decreased over time ($N = 7$, $T = -5.11$, $P \leq 0.01$, $R^2 = 0.839$, equation $y = 79.844\ e^{-0.023x}$). The dissipation rate of niclosamide from the sediment was calculated to be about 2.28% per hour which corresponds to a half-life of 1.27 days. Niclosamide was not detected in control samples.

RESULTS – OBJECTIVE 3

Underwater Camera Footage of a Granular Bayluscide Application

Technical difficulties with the camera system and programming errors resulted in little usable footage. At the St. Clair 1 site, the view was of the silty stream substrate which reflected light back up to the camera and blended into the green water. Footage of the St. Clair 2 site was better quality but the timing was off. Sparse aquatic vegetation (*Potamogeton sp.*) provided a reference point and contrast in the camera image. A few small yellow perch (*Perca flavescens*; Mitchill, 1814) were viewed for about 2 hours following treatment at this site. In general, mostly green water was viewed on the screen at both sites, gB was not visible falling through the water column or on the stream substrate, and larval sea lampreys and non-target organisms were not observed. The cold water reduced the battery life and cameras mounted lower in the water column would have provided a better view of the sediment and contrast with the water. The GoPro® video footage taken by SLCP personnel demonstrated that there were large areas (not quantified) of the St. Clair 2 survey plot that did not have granules on the sediment surface.

DISCUSSION

Niclosamide in Water

Low concentrations of niclosamide were detected in the water column at all depths in all survey plots and grids. The range of concentrations observed at 0.25 h in the survey plots (St. Clair River, 0.08–12.44 $ug \cdot L^{-1}$; Au Train River, 1.34–27.87 $\mu g \cdot L^{-1}$) may be due to the uneven distribution of granules on the sediment surface. GoPro® footage of the St. Clair 2 plot showed large swaths (not quantified) without gB on the sediment surface. This has also been observed by staff that routinely apply gB (Jamie Criger, U.S. Fish and Wildlife Service, Oral Comm., 2018). Uneven gB distribution may introduce areas of higher niclosamide concentration, which could negatively affect non-target species and leave sections uneffectively treated for sea lamprey control.

The sediment composition in the two rivers was very different but there does not appear to be a relation between niclosamide in the water column and TOC measured (Figure 8). One composite sample from each study site was analyzed for TOC. This makes it difficult to correlate niclosamide concentrations in the water and sediment to the TOC values observed in this study. The St. Clair River sediment appeared to have a greater silt and clay content (visual observation) than Au Train River, and little to no sand. Sand, silt, and clay also play a role in niclosamide adsorption (Dawson et al., 1986). Knowledge regarding the influence of these components on niclosamide partitioning, and how they affect the availability of the lampricide to larval sea lampreys, would be enhanced by further investigation.

Niclosamide was expected to dissipate from the water column in proportion to the stream flow at each study site. Stream flow was greater at St. Clair 2 and 3 sites than the St. Clair 1 site (Table 3). However, niclosimide concentrations did not decrease much over time at the St. Clair 2 and 3 sites, and were greater at 24 h than the St. Clair 1 site. It appears that the sediment-sorbed niclosamide continued to desorb into the water column over time to reach equilibrium. The sediment was a reservoir and subsequently, niclosamide continued to be detected in the water 24 hours after gB was applied. This suggests that water flow may influence the amount of niclosamide in the water column and how long it persists in sediment. Water chemistry, particularly pH (Fathulla, 1999), would also play a role in this process as niclosamide is more soluble in water as pH increases. Dawson (1986) found that niclosamide desorbs from the sediment more readily in water of higher pH. The influence of these factors on the behavior of niclosamide in lotic systems is important, however, this study, as designed, could not delineate their influence.

All concentrations in this study were lower than those measured 10 cm above the sediment surface in Seneca Lake [87 $\mu g \cdot L^{-1}$ (+/- 110 SD); Ho and Gloss, 1987] and at two lentic sites on the Little Ausable River delta [258.75 $\mu g \cdot L^{-1}$ (+/- 195.61 SD) and 167.95 $\mu g \cdot L^{-1}$ (+/- 112.65 SD); Gruendling and Bogucki, 1993] after the application of a 5% gB formulation. Bernardy et al. (2020) reported average niclosamide concentrations of 150 $\mu g \cdot L^{-1}$ (+/- 130 SD) at 1 cm and 110 $\mu g \cdot L^{-1}$ (+/- 120 SD) at 13 cm above the sediment surface of lentic survey plots (Lake Michigan) at 0.25 h. The lower concentrations measured in this study, compared to the Lake Michigan lentic sites, could be due to dilution from river flow as well as greater niclosamide partitioning into organic matter and finer clay and silt (Dawson, 1986).

Lake Michigan study sites were located along the shoreline, close to river outlets, and the substrate had a greater sand content. Niclosamide concentrations in survey plot sediment (five sites) for this study averaged 420 $mg \cdot kg^{-1}$ (+/- SD 320) at 8 h, verses 2.9 $mg \cdot kg^{-1}$ (+/- SD 2.4) at 0.25 h and 1.3 (+/- SD 1.8) at 7 h in the 6 lentic plots (Bernardy et al., 2020). Percent TOC averaged 0.82 (0.33–1.34) at sites in this study verses 0.92 (<0.02–4.2) in the lentic sites (Bernardy et al., 2020). When the TOC site with the greatest value (4.2%) is removed, the average TOC of the lentic sites is 0.27. However, niclosamide concentrations at this site averaged 2.53 $mg \cdot kg^{-1}$ (+/- SD 2.91) at hour 0.25 h and 3.30 (3.31) at hour 7, which were not the greatest values of the 6 plots during those sample periods (Bernardy et al., 2020).

Similar to this study, there didn't seem to be a relationship between TOC and niclosamide concentration in the sediment. In contrast, Dawson (1986) reported that sediment with the greatest organic matter (9.0%) had the greatest sorbtion coefficient [68.9 (+/- SD 6.74)], whereas sediment with the least amount of organic matter (0.9%) had the smallest sorbtion coefficient [4.79 (+/- SD 0.39)] at a pH of 8.0. However, their work was conducted in a static laboratory setting. The average TOC in this study (0.82%) was slightly lower than the lowest sediment TOC in Dawson (1986), which was from the Taquamenon River and classified as sandy. The overall low concentrations in all studies mentioned reflect the low solubility of niclosamide in water with chemical parameters typically found in the Great Lakes basin (Fathulla, 1999).

Niclosamide in Sediment

Niclosamide was detected up to 24 h in all survey plot and grid sediment samples, except at 100 m in the Au Train River. Average sediment concentrations were greatest in the 10 m grids and decreased downstream (Tables 6–8, Figure 8). The relatively high concentrations at 10 m could be due to granules drifting downstream (Table 1; Schueller et al., 2018) with the streamflow and from turbulence caused by prop wash (Loberto, 2007). Furthermore, as the 10 cm grid was the closest to the survey plot, niclosamide partitioning (Fathulla, 1999; Dawson, 1986) into the sediment may have occurred to a greater extent than at 30 and 100 m. Partitioning of niclosamide from the water into the sediment as it was carried downstream by the flowing river water is the probable source of niclosamide at 30 and 100 m, rather than the deposition of granules. Relatively high niclosamide concentrations were measured in the 100-m grid at Au Train 2 site during the 0.25 h sample period. This is likely the result of the suspected plume of niclosamide in the water column that flowed from the survey plot and adsorbed to the sediment during this sample period. In addition, some of the plume could have been collected with the sediment sample during this sample period. Concentrations at hour 2 and after were lower and consistent with the other grids (Table 4).

Niclosamide has been shown to dissolve into the water column and sediment pore water, and adsorb to sediment particles, at rates consistent with its limited water solubility (Fathulla, 1999) and the sediment's adsorption capacities (Dawson et al., 1986). Dawson et al. (1986) reported that niclosamide from a Bayer 73 (Bayluscide) application decreased by more than 80% in portions of the Ford River (Michigan) during the 1971 treatment. This resulted in an ineffective treatment and niclosamide adsorption to the sediment was suspected. Further laboratory investigation revealed that niclosamide in fortified water was reduced by 89% when exposed to “black mud rich in organic detritus” and 49% when exposed to a “silt-sand mixture, also rich in organic detritus but more coarse in texture than the black mud” of Ford River sediment (Dawson et al., 1986). In contrast, the niclosamide concentration did not change in the control sample of fortified water with no sediment. Consequently, sediment may act as a reservoir of niclosamide. As concentrations in the water column are diluted by the stream flow, niclosamide desorbs from the sediment (particulate and pore water) in order to maintain equilibrium. This is supported by Dawson (1986) who demonstrated niclosamide desorption of <10% from silt and clay sediment and <30% from sediment with greater sand and lower silt and clay. They postulated that sediment desorption could contribute to lengthening and tailing of treatment blocks in rivers when TFM-1% niclosamide is used. Sediment-sorbed niclosamide was likely the source of the prolonged niclosamide, albeit at low concentrations, in the water column for up to 24 hours in this study.

Management Implications

Theoretically, gB applications deliver 9.275 mg/L (9,275.0 $\mu g \cdot L^{-1}$) in the 5 cm of water above the sediment. This value was derived from the following calculation:

$$\text{Application Rate} = 17{,}500 \text{ mg gB} \cdot m^{-2}$$

$$1 \text{ m}^2 \times 0.05 \text{ m (= bottom 5 cm)} = 0.05 \text{ m}^3 \text{ or } 50 \text{ L}$$

$$\text{gB contains } 2.65\% \text{ niclosamide}$$

$$17{,}500 \text{ mg} \div 50 \text{ L} \times 0.0265 = 9.275 \text{ mg} \cdot L^{-1} \text{ } (9{,}275.0 \text{ } \mu g \cdot L^{-1})$$

This concentration is theorized to provide a lethal dose to larval sea lamprey before it is diluted throughout the water column (Michael Boogaard, U.S. Geological Survey, Written Comm., 2018). Following this calculation, the estimated concentration in the 10 cm of water above the sediment would be 4.6375 mg·L^{-1} (4,637.5 µg·L^{-1}) if all niclosimide instantaneously dissolved into that portion of the water column. The average niclosamide concentration in water taken 10 cm above the sediment surface, in survey plots at 0.25 h, was about three orders of magnitude lower than this value.

It appears that most niclosamide adsorbed to the sediment in the survey plots (Tables 6–8, Figure 8). How exposure to niclosamide-laden sediment and pore water contribute to toxicity is unknown. Previous toxicity studies conducted in the laboratory used larvae that were free swimming or burrowed in sand, and niclosamide concentrations in the sediment were not measured. Larval sea lampreys mostly remain completely buried in the substrate and are passive feeders. They form a tube around the head and brachial region (bladder-like structure) to facilitate water current by adhering silt particles with a mucoid secretion of the endostyle and skin glands (Vladykov, 1952). It is not known whether sediment pore water moves horizontally into the tube. If not, exposure may be a function of the degree to which sediment is pulled vertically into the tube from the water surface. Exposure may also be dependent on how deep larvae are buried. Moore and Beamish (1973) reported that sand and detritus were frequently found in the gut of larvae indicating that some niclosamide exposure could come from the sediment.

It is generally accepted that gB applications provide about a 75% reduction in larval sea lamprey densities (Bills and Genovese, 1990; Fodale et al., 2003). It is not known whether larvae obtain a lethal dose of niclosamide through the water, sediment, or both. The dose received is likely site specific and dependent on gB distribution, sediment composition, water flow, and water chemistry If the sediment is the major route of niclosamide exposure, all factors contributing to uneven distribution of gB such as application, bathymetry, water flow, and boat prop wash turbulence would affect mortality and treatment efficacy. Larvae located in areas of low gB on the sediment surface may not die, and would not die in areas void of gB. If exposure is from the water only, mortality could result from a long and low exposure as niclosamide desorbs from the sediment, provided larvae remain in an area of lethal exposure, that is, where rates of niclosamide uptake exceed the rate of detoxification. This study was designed to evaluate niclosamide concentrations downstream of a gB application to determine the potential exposure to to non-target organisms. We suggest that the SLCP continue to investigate how environmental factors such as streamflow and sediment composition influence gB distribution and niclosamide concentrations at the water-sediment interface. Likewise, further evaluation of application procedures could lead to more uniform distribution of gB on the sediment surface. Knowledge from these investigations could produce more efficacious assessments and treatments.

Bayluscide granules consist of silica sand coated with the ethanolamine salt of niclosamide encapsulated in a water-soluble organic polymer (ethyl and hydroxypropyl cellulose). The polymer delays the release of niclosamide as it sinks through the water to the sediment surface. The polymer is sprayed on using a Wurster Coating Machine (www.wurstercoating.com). However, thickness (target of about 26 µm) varies due to the irregular shape of the sand particles. It is unknown whether niclosamide dissolves into the polymer during the coating process. If this does occur, niclosamide might be released if the polymer begins to dissolve as it sinks through the water column, thereby contributing to a plume. Additionally, niclosamide may release sooner where the polymer layer is thinner on a granule. Abrasion from the blowers and jet propulsion equipment used to apply gB, and prop wash from the boat as it moves through water, as well as the granules as they sink to the substrate, may further accelerate polymer dissolution and niclosamide release. Boogaard et al. (2016a) reported that polymer dissolution and the subsequent niclosamide release time averaged 3.87 min (range 2.20–7.08 min) at 12ºC and 3.38 min (range 1.93–5.40 min) at 21ºC when gB was placed in a petri dish of water. It takes at least 15 minutes to apply gB to a standard larval survey plot (25 × 20 m). Therefore, boat prop wash might also pull niclosamide up from the water-sediment interface after the polymer dissolved. This could help explain why niclosamide was detected at all water depths (0.25 h) in the survey plot and the "plume" measured downstream. Any niclosamide dissolved higher in the water column would detract from the desired lethal concentration required to kill larval sea lampreys at the water-sediment interface. Applications in deeper water may be less impacted by boat prop wash if it does indeed mix niclosamide from the water-sediment interface into the water column.

It is important to note that this study examined the distribution of niclosamide from larval survey plots (25 × 20 m). Treatment plots are typically larger which might result in higher niclosamide concentrations in water and sediment downstream. The dimension and orientation of a plot with respect to the direction of water flow would influence downstream concentrations as well. Also, gB was broadcasted off the front of application boats in the larval survey plots. Applications where gB is applied via jets off the stern of the SLCP's spray boat may yield different results.

Risk to Non-target Organisms

Non-target organisms located between the downstream edge of a survey plot to about 10 m might be at risk if granules settle immediately around or upstream of them. However, exposure to niclosamide via water and sediment alone is expected to be minimal based on the concentrations measured. Granules are not expected to travel beyond 10 m (Table 1) and lower niclosamide concentrations were measured in the water and sediment further downstream. Therefore, exposure and negative impacts to non-target organisms are also expected to be further reduced with increased distance from the survey plot.

Most studies have tested the toxicity of niclosamide to non-target organisms exposed to the liquid formulation of Bayer 73 in laboratory tanks rather than from exposure to gB. Bills and Marking (1976) reported 96 h LC_{50}'s of 28.2 $\mu g \cdot L^{-1}$ for brown trout fingerlings (*Salmo trutta;* Linnaeus, 1758) and 152 $\mu g \cdot L^{-1}$ for bluegill (*Lepomis macrochirus;* Rafinesque, 1819) fingerlings. Marking and Hogan (1967) reported 1 h LC_{50}'s that ranged from 63.0 $\mu g \cdot L^{-1}$ for brown trout to 300 $\mu g \cdot L^{-1}$ for carp (*Cyprinus sp.;* Linnaeus, 1758). They also reported that when exposed to a concentration of 100 $\mu g \cdot L^{-1}$ niclosamide, rainbow trout (*Oncorhynchus mykiss;* Walbaum, 1792) died in 15 minutes, flathead catfish (*Pylodictis olivaris*; Rafinesque, 1818) died within one hour, and yellow perch died within 24 hours. Rye and King

(1976) reported a 24 h LC_{50} of 382.0 $\mu g \cdot L^{-1}$ for the spike freshwater mussel (*Elliptio dilatata;* Rafinesque, 1820) and 34.0 $\mu g \cdot L^{-1}$ for the aquatic earthworm (*Tubifex tubifex*; Müller, 1774) when exposed to Bayer 73. These values are greater than concentrations measured at a depth of 10 cm above the sediment in the water column in all downstream grids of this study. Ho and Gloss (1987) exposed caged fish to gB during a lentic survey and reported 100% survival of largemouth bass (*Micropterus salmoides*; Lacepède, 1802) and 98.7% rainbow trout survival, with the exception of one site that had only 10% survival. It should be noted that the fish in these studies could not swim away from the lampricide exposure.

Boogaard (2016b) reported that after gB was added to tanks, tadpole madtoms (*Noturus gyrinus*; Mitchill, 1817) started to move vertically from 3.5 to 22.5 minutes, and some of the fish remained high in the water column during the entire 60-minute observation period. This demonstrates that risk can be reduced when a non-target organism is able to escape when they detect niclosamide. Exposing freshwater mussels to niclosamide is of particular concern given that they are not mobile and certain concentration and duration combinations can be lethal. However, exposure to mussel species is expected to be low based on the low concentrations measured in this study, especially at distances >10 m downstream from a survey plot.

Preferred larval sea lamprey habitat is primarily of comprised of silt, with sand and detritus as secondary components (USFWS, 2018). The sand fraction is mainly comprised of very fine, fine, and medium sands. Coarse sands, gravel, or rubble may be present, but their contribution is minor (USFWS, 2018). Many of the Great Lakes freshwater mussel species that are state and Federally listed prefer sand-gravel-cobble substrate (Jessica Pruden, U.S. Fish and Wildlife Service, Written Comm., 2022). However, certain mussel species such as the slippershell (*Alasmidonta viridis*; Rafinesque, 1820) and pink papershell (*Potamilus ohiensis*; Rafinesque, 1820) prefer silt-sand-detritus substrates (Jessica Pruden, U.S. Fish and Wildlife Service, Ecological Services, Written Comm., 2022) and may be exposed to niclosamide when granular Balylucide is applied to a plot. The SLCP works with state and Federal agencies to identify and avoid areas where threatened, endangered, and special concern species are known to exist.

Preferred larval habitat in the St. Clair River is a mixture of fine silt and clay, with little to no sand (Aaron Jubar, U.S. Fish and Wildlife Service, Written Comm., 2022). This sediment was ubiquitous on the shallow water shelves of the river and channels. Freshwater mussels are not likely to be present in larval sea lamprey habitat in the St. Clair River and were never observed when collecting sediment for toxicity tests, sampling sediment at study sites, and viewing larval survey sites and the surrounding area. Therefore, exposure of freshwater mussels to niclosimide in survey plots and downstream during gB applications in the St. Clair River is unlikely.

Dissipation Rate

The niclosamide dissipation study (St. Clair River only) was conducted to determine the depletion from sediment over time. The dissipation rate was about 2.28% per hour which corresponds to a half-life of 1.27 days (Table 9, Figure 9). Graebing et al. (2004) demonstrated that niclosamide rapidly degrades by photolysis with a half-life of 4.9 to 5.4 days. Muir and Yarechewski (1982) reported that niclosamide rapidly degraded under aerobic and anaerobic conditions in river and pond sediments at 25ºC with half-life values that ranged from 1.1 to 3.9 days. Dawson (2003) reported that niclosamide is not persistent in the environment and degrades in natural water and sediment systems through hydrolysis, photolysis, and microbial degradation.

Suggested Research

Additional information regarding gB application and efficacy, and how niclosamide behaves in lotic and lentic systems, could improve future assessments and treatments. Research that is likely to provide important insights includes the investigation of (1) niclosamide release time from granules during field applications, (2) polymer coat variation on granules and the value of increased thickness, (3) the role of sediment composition and other related factors that influence niclosamide partitioning, availability, and toxicity/efficacy in the field, (4) the amount of gB required, at a finer scale ($g \cdot m^{-2}$), to achieve desired niclosamide concentrations and larval sea lamprey mortality during field applications, and methods to improve even coverage in a plot, and (5) the survival rate of larval sea lampreys that are captured alive and placed in niclosamide free water following gB applications. Additionally, three-dimentional hydraulic modeling might help answer whether granules are influenced by stream flow and turbulence from boat prop wash.

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DELIVERABLES

1. Data Release
    - Kaye, C.A, J.A. Bernardy, J.R. Schueller, N.A. Schloesser, M.P. Henson, C.K. Andresen, and C.A. Kirkeeng, 2022. Data release for distribution of niclosamide following granular Bayluscide applications in lotic systems. U.S. Geological Survey data release, https://doi.org/10.5066/P9M59ZZU.
2. The Stockbridge High School Robotics Team presented at the Massachusetts Institute of Technology and won an award for their camera design.

RESEARCH HIGHLIGHTS

1. Niclosamide concentrations measured in the water column were much lower than theorized yet detected up to 24 hours.

2. Niclosamide consentrations were consistently greater in the sediment than in the water. The sediment appeared to act as a reservoir whereby niclosamide continuously desorbed over the 24 hour sampling period to maintain equilibrium.

3. GoPro® video footage of one survey plot (St. Clair 2) revealed that there were large areas of river bottom void of gB.

TABLES AND FIGURES

Table 1. Stream sites, site coordinates, mean water depth (m), mean flow rate ($m \cdot sec^{-1}$), estimated time for granular Bayluscide to reach sediment (sec) based on depth and sinking rate (Schueller et al., 2018), and estimated distance (m) granules may have traveled downstream.

Site	Coordinates WGS84 (Decimal Degrees)	Mean Depth (m)	Mean Flow (m·sec-1)	Time to Sediment (sec)	Distance Downstream (m)
Au Train 1	46.432199, -86.823178	1.6	0.15	22.82	3.42
Au Train 2	46.424958, -86.831234	1.0	0.15	14.26	2.14
St. Clair 1	42.574091, -82.662932	1.2	0.16	17.12	2.75
St. Clair 2	42.592410, -82.624465	1.3	0.25	18.54	4.64
St. Clair 3	42.593787, -82.612446	1.1	0.31	15.69	4.86
Dock	42.575383, -82.659961	2.0	-	-	-

Table 2. Water pH and temperature measured in center of the survey plot at each study site in the Au Train (Alger County, Michigan) and St. Clair (St. Clair County, Michigan) rivers following granular Bayluscide application.

Site	pH (si) Temperature (°C)					
	0.25	2	4	6	8	24
Au Train 1	7.63	7.83	8.29	8.43	8.63	7.66
	22.8	23.6	24.4	25.4	26.1	24.6
Au Train 2	7.95	7.86	8.49	9.11	9.10	7.66
	24.1	24.9	26.5	27.5	28.6	26.7
St. Clair 1	8.20	8.10	8.14	8.12	8.13	8.18
	14.3	15.2	14.8	15.6	15.3	14.5
St. Clair 2	8.11	8.17	8.18	-[1]	-[1]	8.07
	16.3	61.4	16.8	-[1]	-[1]	14.2
St. Clair 3	8.12	8.16	8.18	8.10	8.18	8.30
	15.2	15.3	15.8	15.9	16.1	14.9

[1] Samples not taken due to inclement weather and rough seas.

Table 3. Mean niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) concentration [$\mu g \cdot L^{-1}$ (SD)] in the water column at three depths [surface, ½ the water column (WC) depth, 10 cm above the sediment], in five survey plots and downstream grids (10, 30, 100 m) for the Au Train (Alger County, Michigan) and St. Clair (St. Clair County, Michigan) rivers combined following granular Bayluscide application.

Sample Location	Sample Position	Mean Niclosamide Concentration $\mu g \cdot L^{-1}$ (SD)					
		0.25	2	4	6	8	24
Survey Plot	Surface	0.038 (0.072)	0.027 (0.033)	0.039 (0.039)	0.048 (0.050)	0.016 (0.026)	0.020 (0.025)
	½ WC	0.94 (1.50)	0.086 (0.16)	0.057 (0.058)	0.076 (0.080)	0.062 (0.089)	0.073 (0.21)
	10 cm	4.90 (7.20)	0.72 (1.3)	0.38 (0.61)	0.43 (0.88)	0.71 (1.8)	0.063 (0.071)
10 m	Surface	0.17 (0.29)	0.024 (0.024)	0.077 (0.13)	0.052 (0.052)	0.039 (0.066)	0.017 (0.025)
	½ WC	0.53 (0.53)	0.16 (0.13)	0.084 (0.054)	0.096 (0.11)	0.074 (0.10)	0.018 (0.027)
	10 cm	1.6 (1.6)	0.39 (0.48)	0.20 (0.28)	0.46 (0.78)	0.19 (0.28)	0.034 (0.046)
30 m	Surface	0.63 (0.99)	0.10 (0.10)	0.061 (0.049)	0.058 (0.046)	0.026 (0.031)	0.0089 (0.011)
	½ WC	1.1 (1.3)	0.12 (0.13)	0.094 (0.078)	0.08 (0.072)	0.047 (0.049)	0.010 (0.011)
	10 cm	1.3 (1.3)	0.15 (0.16)	0.10 (0.10)	0.16 (0.22)	0.062 (0.062)	0.010 (0.011)
100 m	Surface	2.4 (4.5)	0.082 (0.090)	0.063 (0.043)	0.068 (0.066)	0.018 (0.019)	0.030 (0.049)
	½ WC	2.1 (3.4)	0.11 (0.10)	0.048 (0.033)	0.052 (0.054)	0.041 (0.061)	0.011 (0.015)
	10 cm	2.4 (4.4)	0.24 (0.42)	0.056 (0.050)	0.083 (0.12)	0.037 (0.055)	0.027 (0.048)

Table 4. Mean niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) concentrations [$\mu g \cdot L^{-1}$ (SD)] in the water column at three depths [surface, ½ the water column (WC) depth, 10 cm above the sediment] in two survey plots and downstream grids (10, 30, 100 m) in the Au Train River (Alger County, Michigan) following granular Bayluscide application.

Sample Location	Sample Position	Mean Niclosamide Concentration $\mu g \cdot L^{-1}$ (SD)					
		0.25	2	4	6	8	24
Survey Plot	Surface	0.048	0.00	0.00	0.00	0.00	0.00
		(0.11)	-	-	-	-	-
	½ WC	1.2	0.10	0.023	0.018	0.024	0.00
		(1.2)	(0.24)	(0.055)	(0.044)	(0.059)	-
	10 cm	8.4	1.5	0.71	0.67	1.3	0.066
		(9.8)	(1.9)	(0.91)	(1.3)	(2.6)	(0.10)
10 m	Surface	0.25	0.0028	0.10	0.0044	0.0048	0.00
		(0.41)	(0.0069)	(0.22)	(0.011)	(0.012)	-
	½ WC	0.89	0.27	0.079	0.075	0.041	0.00
		(0.59)	(0.14)	(0.081)	(0.16)	(0.099)	-
	10 cm	3.1	0.84	0.37	0.34	0.33	0.010
		(1.4)	(0.46)	(0.37)	(0.41)	(0.36)	(0.016)
30 m	Surface	1.4	0.16	0.049	0.027	0.019	0.00
		(1.3)	(0.13)	(0.064)	(0.050)	(0.031)	-
	½ WC	2.2	0.23	0.12	0.057	0.028	0.00
		(1.5)	(0.14)	(0.12)	(0.098)	(0.045)	-
	10 cm	2.3	0.28	0.15	0.098	0.043	0.00
		(1.3)	(0.20)	(0.15)	(0.11)	(0.059)	-
100 m	Surface	5.7	0.16	0.037	0.045	0.0064	0.00
		(5.9)	(0.094)	(0.037)	(0.091)	(0.012)	-
	½ WC	4.6	0.16	0.022	0.0021	0.001	0.00
		(4.4)	(0.096)	(0.030)	(0.0052)	(0.0025)	-
	10 cm	5.3	0.22	0.019	0.00	0.00	0.00
		(6.0)	(0.14)	(0.030)	-	-	-

Table 5. Mean niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) concentrations [$\mu g \cdot L^{-1}$ (SD)] in the water column at three depths [surface, ½ the water column (WC) depth, 10 cm above the sediment], in three assessment plots and downstream grids (10, 30, 100 m) in the St. Clair River (St. Clair County, Michigan) following granular Bayluscide application.

Sample Location	Sample Position	Mean Niclosamide Concentration $\mu g \cdot L^{-1}$ (SD)					
		0.25	2	4	6	8	24
Survey Plot	Surface	0.032 (0.034)	0.044 (0.031)	0.061 (0.032)	0.095 (0.010)	0.033 (0.030)	0.028 (0.026)
	½ WC	0.77 (1.8)	0.076 (0.075)	0.080 (0.050)	0.13 (0.064)	0.10 (0.10)	0.12 (0.27)
	10 cm	2.8 (3.8)	0.24 (0.15)	0.16 (0.061)	0.20 (0.069)	0.088 (0.078)	0.061 (0.045)
10 m	Surface	0.12 (0.17)	0.038 (0.020)	0.062 (0.034)	0.099 (0.021)	0.074 (0.081)	0.028 (0.026)
	½ WC	0.29 (0.34)	0.084 (0.064)	0.089 (0.025)	0.12 (0.042)	0.11 (0.10)	0.033 (0.029)
	10 cm	0.58 (0.48)	0.088 (0.092)	0.076 (0.061)	0.23 (2.0)	0.056 (0.063)	0.051 (0.053)
30 m	Surface	0.13 (0.12)	0.063 (0.055)	0.069 (0.039)	0.088 (0.0070)	0.032 (0.033)	0.015 (0.011)
	½ WC	0.30 (0.30)	0.041 (0.014)	0.072 (0.023)	0.10 (0.026)	0.066 (0.049)	0.017 (0.0089)
	10 cm	0.59 (0.66)	0.068 (0.039)	0.076 (0.029)	0.23 (0.28)	0.081 (0.064)	0.016 (0.011)
100 m	Surface	0.21 (0.35)	0.028 (0.020)	0.089 (0.031)	0.092 (0.0059)	0.030 (0.018)	0.050 (0.056)
	½ WC	0.39 (0.59)	0.083 (0.099)	0.068 (0.019)	0.10 (0.023)	0.080 (0.067)	0.018 (0.015)
	10 cm	0.53 (0.48)	0.16 (0.24)	0.084 (0.044)	0.15 (0.12)	0.073 (0.058)	0.045 (0.056)

Table 6. Mean niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) concentrations in sediment [$\mu g \cdot kg^{-1}$ (SD)] in five assessment plots and downstream grids (10, 30, 100 m), in the Au Train (Alger County, Michigan) and St. Clair (St. Clair County, Michigan) rivers combined following granular Bayluscide application.

Sample Location	Mean Niclosamide Concentration $\mu g \cdot kg^{-1}$ (SD)					
	0.25	2	4	6	8	24
Survey Plot	-[1]	-[1]	-[1]	-[1]	420.0[2] (320.0)	310.0[3] (320.0)
10 m	79.0 (200.0)	22.0 (41.0)	7.80 (23.0)	50.0 (56.0)	42.0 (81.0)	34.0 (52.0)
30 m	0.37 (0.71)	6.0 (13.0)	1.1 (3.1)	1.3 (3.5)	1.7 (4.1)	1.1 (2.0)
100 m	2.0 (3.2)	0.24 (0.43)	0.50 (1.2)	0.25 (0.28)	0.39 (0.82)	0.11 (0.12)

[1] Samples not collected to not influence niclosamide concentrations in the downstream grids.

[2] 8 h samples collected in the assessment plot between the downstream edge to 6.25 m upstream.

[3] 24 h samples collected in the assessment plot between 6.25 and 12.5 m up from the downstream edge.

Table 7. Mean niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) concentrations [$\mu g \cdot kg^{-1}$ (SD)] in the sediment in three assessment plots and downstream grids (10, 30, 100 m), in the Au Train River (Alger County, Michigan) following granular Bayluscide application.

Sample Location	Mean Niclosamide Concentration $\mu g \cdot kg^{-1}$ (SD)					
	0.25	2	4	6	8	24
Survey Plot	-[1]	-[1]	-[1]	-[1]	510[2] (400)	280[3] (400)
10 m	51.0 (67.0)	41.0 (60.0)	14.0 (31.0)	49.0 (66.0)	81.0 (100.0)	38.0 (62.0)
30 m	0.87 (0.96)	15.0 (17.0)	0.44 (0.83)	2.1 (4.5)	2.9 (5.7)	0.07 (0.12)
100 m	4.5 (3.8)	0.24 (0.19)	0.0076 (0.013)	0.11 (0.12)	0.095 (0.21)	0.00 -

[1] Samples not collected to not influence niclosamide concentrations in the downstream grids.

[2] 8 h samples collected in the assessment plot between the downstream edge to 6.25 m upstream.

[3] 24 h samples collected in the assessment plot between 6.25 and 12.5 m up from the downstream edge.

Table 8. Mean niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) concentrations [$\mu g \cdot kg^{-1}$ (SD)] in sediment, in three survey plots and downstream grids (10, 30, 100 m), in the St. Clair River (St. Clair County, Michigan) following granular Bayluscide application.

Sample Location	Mean Niclosamide Concentration $\mu g \cdot kg^{-1}$ (SD)					
	0.25	2	4	6	8	24
Survey Plot	-[1]	-[1]	-[1]	-[1]	310.0[2] (160.0)	330.0[3] (270.0)
10 m	97.0 (260.0)	9.8 (16.0)	62.0 (0.75)	53.0 (46.0)	3.1 (2.5)	32.0 (52.0)
30 m	0.037 (0.036)	0.035 (0.038)	1.7 (4.2)	0.17 (0.081)	0.54 (0.83)	1.5 (2.2)
100 m	0.31 (0.53)	0.25 (0.55)	0.78 (1.5)	0.40 (0.35)	0.63 (1.1)	0.15 (0.11)

[1] Samples not collected to not influence niclosamide concentrations in the downstream grids.

[2] 8 h samples collected in the assessment plot between the downstream edge and 6.25 m upstream.

[3] 24 h samples collected in the assessment plot between 6.25 and 12.5 m up from the downstream edge.

Table 9. Sample tray, time (h) deployed in river, percent niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) recovered, and mean niclosamide concentration [$\mu g \cdot kg^{-1}$ (SD)] in glass jars (three per replication/tray) dosed with granular Bayluscide at the field application rate (0.0496 g) and placed on the sediment surface in the St. Clair River (St. Clair County, Michigan).

Time (h)	Sample Tray	% Recovery (SD)	Concentration $\mu g \cdot kg^{-1}$ (SD)
0.00	H	88.4 (3.6)	2.23 (0.088)
0.02	A	87.3 (8.5)	2.20 (0.21)
0.98	B	66.8 (6.2)	1.68 (0.15)
1.02	I	93.2 (6.8)	2.32 (0.19)
1.95	J	93.1 (6.4)	2.33 (0.16)
2.05	C	70.8 (14.1)	1.78 (0.34)
3.98	K	80.7 (13.7)	3.30 (2.21)
4.02	D	50.1 (10.3)	1.26 (0.27)
6.00	E	57.8 (22.2)	1.46 (0.57)
6.02	L	75.9 (21.8)	1.90 (0.54)
7.98	F	60.4 (18.8)	1.52 (0.47)
7.98	M	22.0 (3.9)	0.55 (0.10)
23.88	N	36.8 (16.4)	0.92 (0.41)
23.97	G	60.8 (20.1)	1.53 (0.49)

Figure 1. Location of the two disspation study sites (red tear drops) in the Au Train River (Alger County, Michigan).

Figure 2. Location of the four study sites in the St. Clair River (St. Clair County, Michigan). Red tear drops depict the three disspation study sites and the green arrow depicts the location of the dock where the niclosamide depletion study occured.

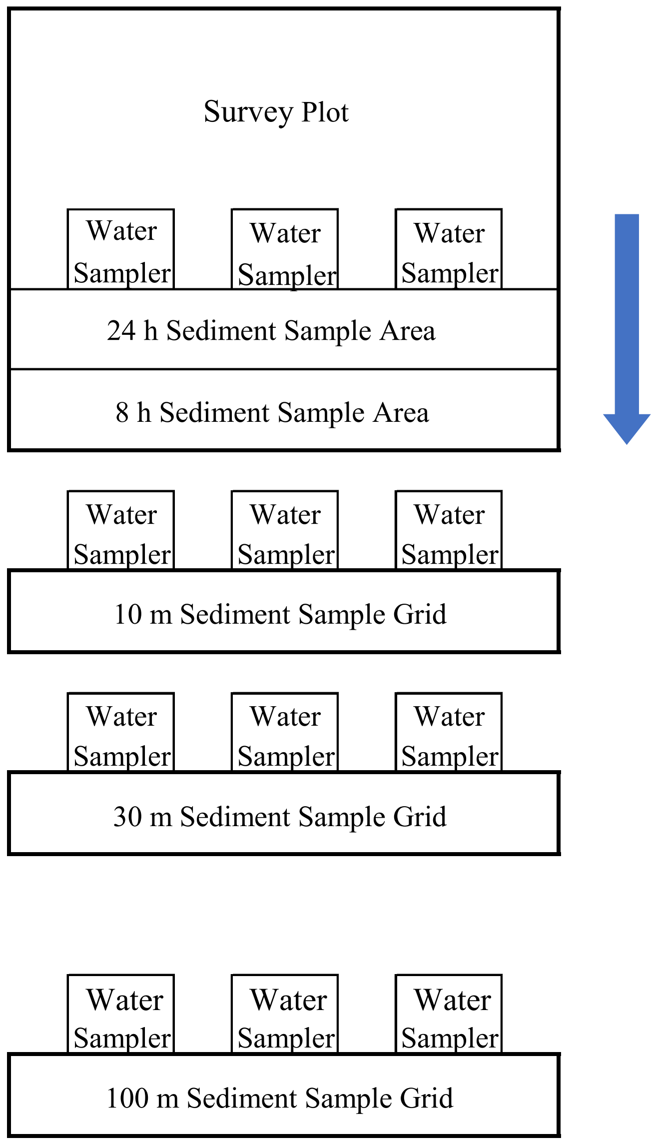

Figure 3. Study site design in the Au Train (Alger County, Michigan) and St. Clair (St. Clair County, Michigan) rivers. Each site had a survey plot, sample grids located 10, 30, and 100 m downstream, and 12 water samplers. Blue arrow indicates water flow.

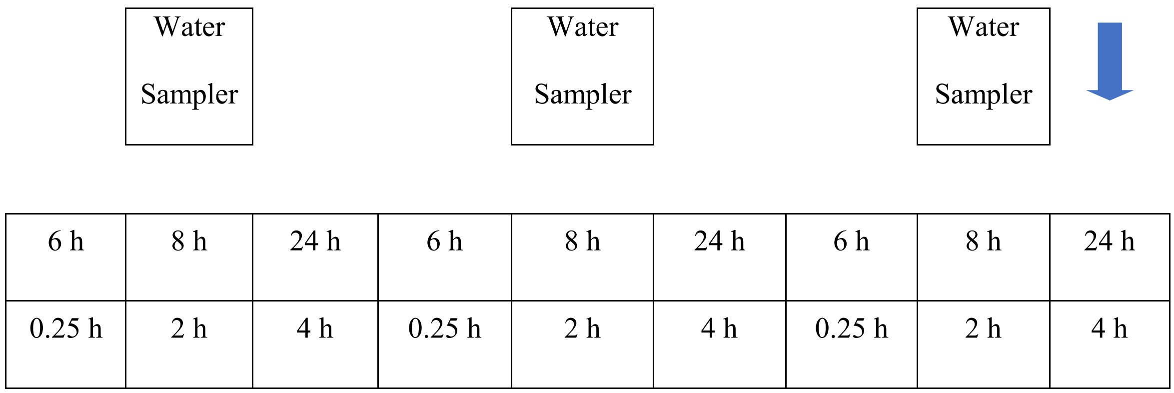

Figure 4. Design of sample grids located 10, 30, and 100 m downstream from survey plots in the Au Train (Alger County, Michigan) and St. Clair (St. Clair County, Michigan) rivers. Each grid (6 m wide upstream /downstream × 18 m long) had two rows of nine sample sections (3 m wide upstream/downstream × 2 m long). Numbers indicate the pre-designated sample periods. Blue arrow indicates water flow.

Figure 5. Example of the dissipation study design at Site 2 in the St. Clair (St. Clair County, Michigan). The red square depicts the survey plot. The orange rectangles depict the sample grids placed 10, 30, and 100 m downstream of the plot. The blue arrow indicates water flow.

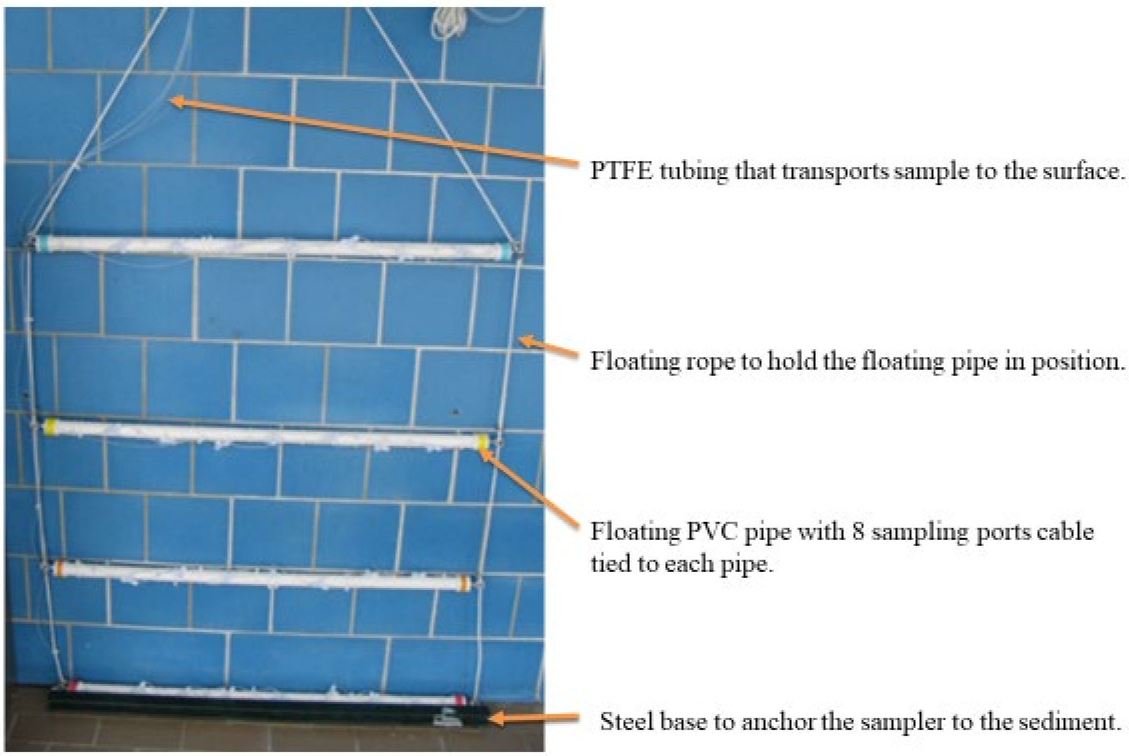

Figure 6. Sampler used to collect water in survey plots and upstream of sample grids at 10 cm above the sediment and ½ the water depth (50-80 cm) from 8 ports that were equally spaced across the 1 m PVC pipe.

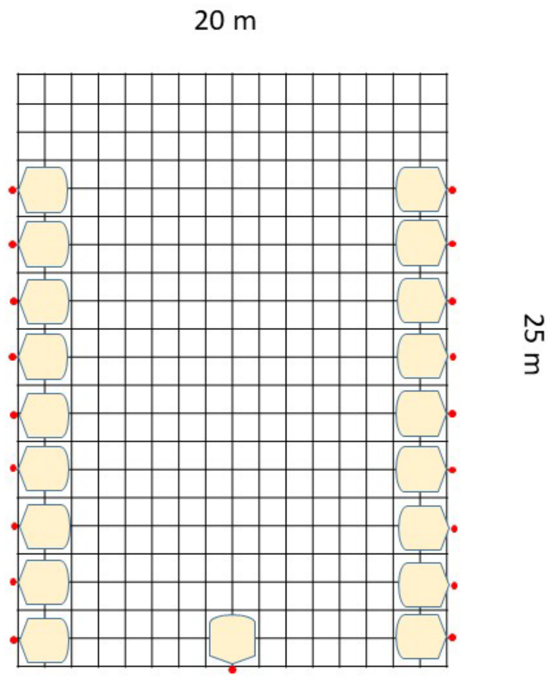

Figure 7. Camera setup in two St. Clair River (St. Clair County, Michigan) survey plots (25 m long upstream/downstream × 20 m wide). Red dots represent placement of underwater cameras and yellow squares represent the camera view (1.22 × 1.22 m).

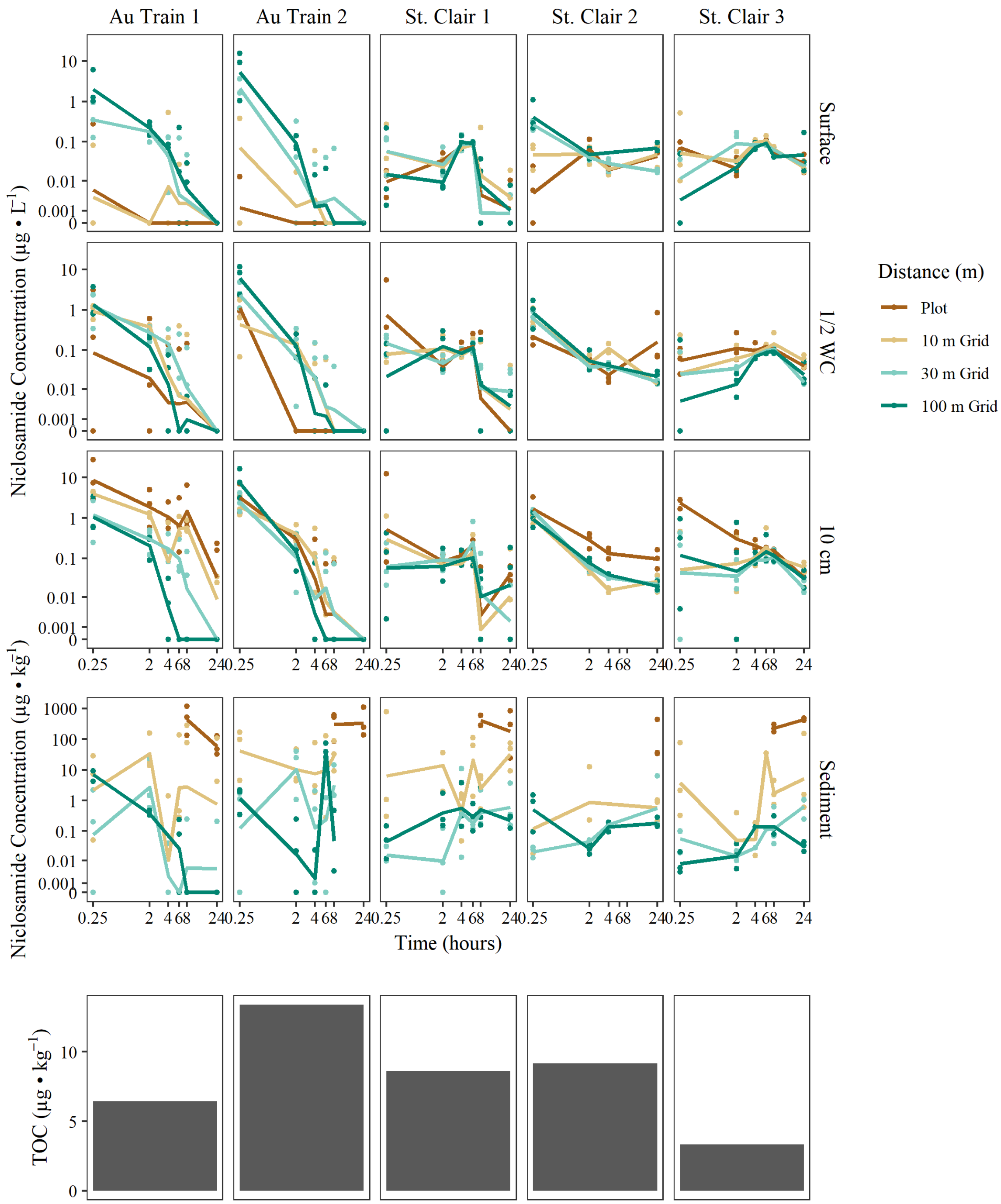

Figure 8. Niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) concentrations (log scale, µg·L^{-1}) at three depths in the water column (10 cm above the sediment, ½ water depth, water surface) and sediment (log scale, µg·kg^{-1}) in five survey plots and downstream grids (10, 30, 100 m) in the Au Train (Alger County, Michigan) and St. Clair (St. Clair County, Michigan) rivers at 0.25, 2, 4, 6, 8, and 24 h after granular Bayluscide application. The time scale was log-transformed to better visualize the data. Total organic carbon (TOC, µg·kg^{-1}) of sediment composite measured for each study site.

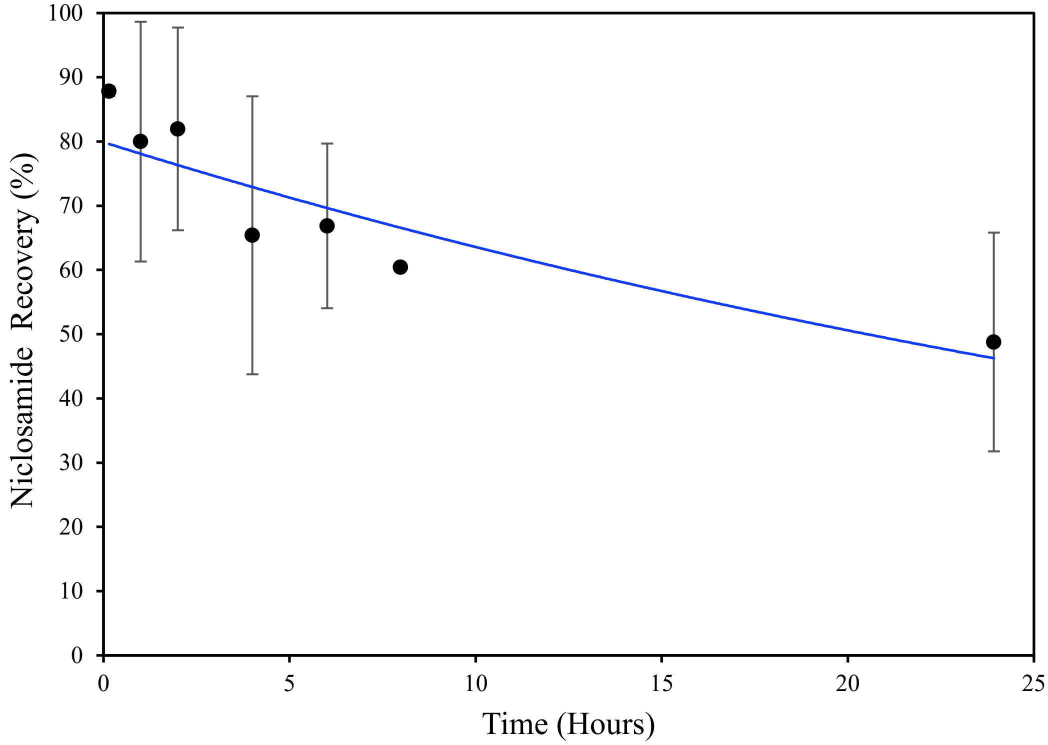

Figure 9. Percent niclosamide (2′,5-dichloro-4′-nitrosalicylanilide) recovered in St. Clair River sediment over 24 h. Error bars represent the standard deviation between two trays retrieved during each sample period. One 8 h sample tray was compromised and removed from the dataset.