# GREAT LAKES FISHERY COMMISSION

## 2020 Project Completion Report[1]

## Distribution of Niclosamide Following Granular Bayer Applications in Lentic Environments

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[*August 2020*]

[1] Project completion reports of Commission-sponsored research are made available to the Commission's Cooperators in the interest of rapid dissemination of information that may be useful in Great Lakes fishery management, research, or administration. This product has been reviewed according to U.S. Geological Survey (USGS) Fundamental Sciences Practices (https://pubs.usgs.gov/circ/1367/), which includes USGS-coordinated peer review. Sponsorship of the project by the Commission does not necessarily imply that the findings or conclusions are endorsed by the Commission.

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**ABSTRACT**

Temporal and spatial distribution of niclosamide in the water column and sediment were evaluated after the application of granular Bayluscide in six lentic sea lamprey (*Petromyzon marinus*) larval assessment plots. Water and sediment were collected 0.25, 1, 3, 5, and 7 hours after application and were analyzed for niclosamide, the active ingredient in granular Bayluscide. Water samples were collected from five heights in the water column (1 cm, 13 cm, 26 cm, ½ water column, and water surface) at five locations inside and four locations 10 m outside of each assessment plot. Sediment was collected from 18 locations within each plot. Niclosamide water concentrations inside and outside of the plots did not vary by depth but did vary between plots and by time. Niclosamide water concentrations also varied by sampler location outside of the plots. Following granular Bayluscide applications the mean niclosamide concentration in water for all levels, within the plots, decreased from 0.12 $mg \cdot L^{-1}$ (SD = 0.12 $mg \cdot L^{-1}$) at 15 minutes to 0.061 $mg \cdot L^{-1}$ (SD = 0.040 $mg \cdot L^{-1}$) at hour 1. The mean niclosamide concentration in the top 4 cm of sediment was 2.9 $mg \cdot kg^{-1}$ (SD = 2.4 $mg \cdot kg^{-1}$) 15 minutes after application and was 1.3 $mg \cdot kg^{-1}$ (SD = 1.8 $mg \cdot kg^{-1}$) at hour 7. Concentrations in all sediment samples ranged from < 0.001 to 30.730 $mg \cdot kg^{-1}$ and varied between the six plots. Niclosamide concentrations measured in sediment samples were more than 1 order of magnitude greater than in the water and varied spatially by over 4 orders of magnitude.

## INTRODUCTION

Sea lampreys (*Petromyzon marinus*) are controlled in the Great Lakes using two lampricides, 3-trifluoromethyl-4-nitrophenol (TFM) and Bayluscide® (Dawson 2003; Hubert 2003). The granular formulation of Bayluscide (3.2% Granular Sea Lamprey Larvicide; granular Bayluscide) is used to treat larvae in deep or large lotic systems where the use of TFM is ineffective or prohibitively expensive, and in lentic areas. Granular Bayluscide is applied per the product label and Sea Lamprey Control Program's (SLCP) Technical Operating Procedure (TOP):017 *Procedure for Application of Bayluscide 3.2% Granular Sea Lamprey Larvicide for assessment or control applications* (Solomon 2019) at a rate of 17.5 $g \cdot m^{-2}$, irrespective of water pH, depth, and flow rate. It is broadcast over the water surface with custom application boats to ensure coverage over a designated area. The granules consist of silica sand coated with the ethanolamine salt of niclosamide (5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide) encapsulated in a water-soluble organic polymer. The polymer coating breaks open within 3 to 4 minutes after entering the water and exposes the niclosamide (Boogaard et al. 2016).

Previous research provides insight on the fate and distribution of niclosamide in aqueous environments; however, there are no data on the concentration of niclosamide near the sediment-water interface or in the sediment where larval lamprey burrow. Two studies were conducted in lentic environments that measured the niclosamide concentrations in water following the application of a 5% granular Bayluscide formulation. Ho and Gloss (1987) measured niclosamide concentrations at 9 sites in Seneca Lake (New York) from 10 cm above the sediment to the water surface for 96 hours. The mean concentration at 10 cm was 0.071 $mg \cdot L^{-1}$ (SD = 0.086 $mg \cdot L^{-1}$) 1 hour after granular Bayluscide application. Gruendling and Bogucki (1993) measured niclosamide concentrations 10 cm above the sediment at 21 sites for 98 hours in the Little Ausable River (New York). The mean concentration was 0.21 $mg \cdot L^{-1}$ (SD = 0.16 $mg \cdot L^{-1}$) 3 hours after application. Neither study sampled the bottom 5 cm of the water column or the sediment.

Other studies have examined binding, movement, and degradation of niclosamide within sediment. Dawson et al. (1986) reported that niclosamide could leach more than 25 cm in sandy sediment at pH 9

but did not leach in loamy sand and finer sediments in water pH ≤ 8. Laboratory studies indicate that the half-life of niclosamide in sediment with water varies from 1.1 to 3.9 days (Muir and Yarechewski 1982) and 4.9 to 5.4 days (Graebing et al. 2004).

Granular Bayluscide applications are essential to the SLCP. Water currents, water depth, wind, waves, and aquatic vegetation are known to affect the efficacy of treatments and assessments (Solomon 2019). A better understanding of niclosamide concentrations and distribution in the water column and sediment following application could lead to improved efficacy and a better understanding of the potential risk to non-target organisms.

## OBJECTIVE

The objective of this study was to determine the distribution of niclosamide in the water column and sediment over time in lentic systems following granular Bayluscide applications. The study objective was met, and we found a mean niclosamide concentration of 0.054 $mg \cdot L^{-1}$ relatively evenly dispersed in the water column and a mean concentration of 2.1 $mg \cdot kg^{-1}$ not evenly distributed in sediments.

## METHODS

### *Assessment plot locations and study chronology*

Assessment plots were located in the bay at the mouth of Hog Island Creek (Lake Michigan, Mackinac County, Michigan) and in Peshtigo Harbor at the mouth of the Peshtigo River (Lake Michigan, Marinette County, Wisconsin). The location of each plot was determined following consultation with SLCP larval assessment personnel (Table 1). Plots were 20-m wide by 25-m long in water 0.52 m to 1.2 m deep. The side of each plot (20-m) closest to and parallel to the shoreline was designated as Land (Figure 1). The other (20-m) side, farther out into the lake, was designated as Lake. The sides (Left and Right) were designated by looking toward the Lake side from the Land side. Each plot was setup and control samples were collected the day prior to granular Bayluscide application (Table 2). Water temperature and pH were measured near the surface next to water samplers A, C, and E approximately 15–30 minutes before

granular Bayluscide was applied to each plot (Table 2). Larval assessment personnel applied 8.75 kg granular Bayluscide as prescribed by TOP:017 *Procedure for Application of Bayluscide 3.2% Granular Sea Lamprey Larvicide for assessment or control applications* (Solomon 2019) by maneuvering from side to side through the plot between the rows of samplers. Time 0 started immediately upon completion of the application. Water (n = 25) and sediment (n = 18) samples were collected periodically (0.25, 1, 3, 5, and 7 h) after application. Temperature and pH of the sediment was measured in the first 3 control and last 3 test trays retrieved (Table 3).

*Water samples*

Water samplers (A–E) were placed at 2, 6, 10, 14, and 18 m across the width and 5 m inside the Lake edge of the plot (Figure 1). Water samplers (F–I) were placed 10 m outside, parallel to, and centered along each side of the plot. Each sampler was anchored to the sediment with a 1-m long, 15-cm wide, and 7-mm thick piece of steel and drew water from four depths (1, 13, and 26 cm above the sediment, and 1/2 water column) from eight evenly spaced intake ports fixed to 1-m lengths of horizontally floating pipe (Figure 2). All water lines were 3.63–3.69 m long and made from 1.6 mm inside diameter polytetrafluoroethylene (PTFE) tubing (total volume of each line was 7.2–7.3 mL). Water (25–28 mL) was drawn through each line and discarded before collecting a sample to eliminate niclosamide carryover between samples.

Control and test water samples were collected via kayak in the following order: surface, 1 cm, 13 cm, 26 cm, and ½ water column. Surface water was collected by placing the end of a new 3-mL syringe (part number 309657 Becton, Dickinson and Co., Franklin Lakes, New Jersey) roughly 2 cm below the water surface, drawing a sample, and transferring a 1-mL aliquot to two prelabeled cryogenic vials (part number 1420-8300; USA Scientific Inc., Ocala, Florida). The lower four levels were collected by (1) attaching a 30-mL syringe (part number 302832 Becton, Dickinson and Co., Franklin Lakes, New Jersey) to the appropriate sample line, (2) drawing 25–28 mL of water through the line to remove all carryover, (3) closing the stopcock at the end of the line, (4) replacing the 30-mL syringe with a single use 3-mL

syringe, (5) opening the stopcock, (6) drawing 3 mL of sample into the syringe, (7) closing the stopcock, (8) removing the syringe and (9) transferring 1 mL of the water sample into two prelabeled cryogenic vials. For quality control and storage stability assessments, 50-mL centrifuge tubes (part number 430290; Corning Inc., Corning, New York) were filled with river water near water sampler C. In the field and immediately preceding analysis in the lab, five volumetric flasks (5-mL), fortified with 100 µL of 100 $\mu g \cdot mL^{-1}$ niclosamide, were filled to volume with the river water from the 50-mL tubes, mixed, and transferred to 1.2-mL cryogenic microcentrifuge vials. The ratios between the field and lab fortified recoveries were used to correct the samples for loss during storage. All water samples were frozen (-20 °C) in the field, then held at (-80 °C) at the U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) until analyzed.

Water samples were prepared for analysis by warming them to ambient room temperature and then mixing by inversion. Each sample was centrifuged at 15,000 relative centrifugal force for 10 minutes at 20 °C (Avanti 30; Beckman Coulter, Inc., Brea, California). Approximately 500 µL of supernatant was transferred to a liquid chromatography vial (part number 600000669CV; Waters Corp., Milford, Massachusetts). Niclosamide in the water samples was quantified at UMESC using the methods described in the niclosamide quantification section. Water sample data collected for this study are available in Bernardy et al. (2020).

*Sediment Samples*

Sediment samplers were lowered to the lake bottom from kayaks in six rows spaced 3 m apart (Figure 3). Each row started 2 m in from the perimeter of the plot and had 9 trays placed 2 meters apart. Each sediment sampler consisted of two 4-cm deep x 11-cm diameter dishes placed in a 5-cm deep x 15-cm wide x 23-cm long tray (Figure 4). Fishing line attached to the corners of each tray and to a labeled float were used to deploy, locate, and retrieve the samplers. Sediment collected from the site and homogenized in 5-gallon buckets was used to fill the dishes (n=108). Two 1-L subsamples were placed in half-gallon glass mason jars, frozen at -20 °C in the field, and stored at -80 °C until analyzed for total organic carbon

by a contract laboratory (Davy Inc., La Crosse, Wisconsin; method MSA 29-3.5.2 (Page et al. 1982); Table 3).

Nine preassigned sediment samplers were collected throughout the plot at each time period (Figure 1) by slowly pulling each tray to the surface where sediment was placed in individual tared, quart-size freezer bags, sealed, and mixed by hand for 1-1.5 minutes. Small pin holes were made in a bottom corner of each bag and pore water (approximately 5 mL) was squeezed into a prelabeled 5-mL graduated amber polypropylene vial. The remaining pore water was measured with a graduated cylinder, then discarded. The sediment particulate fraction of the sample was weighed and mixed again for 1–1.5 minutes. Duplicate subsamples (15–20 g) were transferred from the bag into prelabeled and tared 50-mL centrifuge tubes. High pressure liquid chromatography-grade methanol (10 mL) was added to each tube, the tube capped, and then mixed by inversion. For quality control and storage stability assessments, 5 of the 18 control sediment samples collected were fortified as follows: (1) the sediment particulate and pore water fractions were separated as above, (2) five of the particulate fractions were fortified with 167.7 mg of granular Bayluscide and mixed for 1–1.5 minutes, (3) after 15 minutes, the samples were mixed a second time for 1–1.5 minutes, and (4) triplicate 15–20 g subsamples were stored in 50-mL centrifuge tubes with 10 mL methanol. One control sample was separated into particulate and pore water fractions then the particulate fraction was divided into 12 subsamples (15–20 g) and 9 of the subsamples were fortified in triplicate with 100 $\mu g \cdot mL^{-1}$ niclosamide standard. The other 3 subsamples were fortified in the lab. Control pore water was fortified with 100 µL of 100 $\mu g \cdot mL^{-1}$ niclosamide in five 5-mL volumetric flasks, brought to volume and transferred to 1.2-mL microcentrifuge tubes. A minimum of three 15-mL centrifuge tubes were filled with pore water for determination of lab fortified sample recovery. All particulate fractions were stored at -20 °C in the field, transferred on wet ice to the UMESC, and stored at -80 °C until analyzed for niclosamide. All pore water fractions were frozen (-20 °C) in the field, then held at (-80 °C) at the UMESC until analyzed.

Particulate fractions were prepared for analysis by warming to ambient room temperature. Niclosamide was extracted from each sample with methanol as follows: (1) add 10 mL of methanol, (2) agitate for 5 minutes (Multi-Wrist Shaker model 3589; Lab-Line Instruments, Inc., Melrose Park, Illinois), (3) centrifuge at 5,000 relative centrifugal force for 5 minutes at 20 °C (Avanti model J-26 XPI; Beckman Coulter, Inc., Brea, California), (4) transfer supernatant to a 50-mL volumetric flask, and (5) repeat two more times transferring the supernatant into the same flask. E-pure water (20 mL) was added to the supernatant and mixed. The sample was cooled to 20 °C then brought to volume with methanol and mixed. A 1-mL subsample was either filtered through a 0.2-µm regenerated cellulose filter (Phenex RC membrane; Phenomenex Co., Torrance, California) or transferred to a 1.2-mL microcentrifuge tube and centrifuged at 10,000 relative centrifugal force for 10 minutes at 20 °C (Avanti model 30; Beckman Coulter, Inc., Brea, California). Approximately 500 µL of the supernatant was transferred to a liquid chromatography vial.

Pore water fractions were prepared for analysis by warming to ambient room temperature and mixing by inversion. Pore water (1 mL) was transferred to a 1.2-mL microcentrifuge tube and centrifuged at 15,000 relative centrifugal force for 14 minutes at 20 °C using a Beckman Instruments Avanti model 30 centrifuge. Approximately 500 µL of the supernatant was transferred to a liquid chromatography vial. Niclosamide in the sediment samples was quantified using the methods described in the niclosamide quantification section. Sediment sample data collected for this study are available in Bernardy et al. (2020).

*Niclosamide Quantification*

A binary Agilent model 1260 liquid chromatograph (Agilent Technologies, Inc., Santa Clara, California) system equipped with a Kinetex, 2.6 µ, XB-$C_{18}$, 50 x 2.1 mm liquid chromatography column (Phenomenex Co., Torrance California) and an Agilent model 6460 triple quadrupole mass detector with an Agilent Jet Stream electrospray ionization source was used to measure the niclosamide in the samples. The injection volume for all samples and standards was 2 µL. Seven niclosamide calibration curve

standards (0.0027-2.0000 $\mu g \cdot mL^{-1}$) and all samples were chromatographed in 50 °C mobile phase (A = 750 mL e-pure water + 250 mL mass spectrometry grade methanol + 385 mg mass spectrometry grade ammonium acetate; B = 1000 mL methanol + 385 mg ammonium acetate) flowing at 0.60 $mL \cdot minute^{-1}$. A 1.5-minute mobile phase gradient starting at 45.0% B and switching to 70.0% B at 0.0 minute and back to 45.0 % B at 0.5 minute was used to elute niclosamide.

The source operated in negative mode under the following parameters: gas (nitrogen, 350 °C and 9.0 $mL \cdot minute^{-1}$), nebulizer (25 pounds per square inch), sheath gas (nitrogen, 375 °C and 11.0 $L \cdot minute^{-1}$), capillary (-3000 volts) and nozzle (-500 volts). The fragmentor voltage was set at 115 and transitions 325→289 and 325→171 were monitored. Samples that did not fit within the calibration curve were diluted with a 60% methanol/40% water solution and reinjected. Calibration standards were considered acceptable for use only if two independently prepared sets of standards were within 5% of each other. Each sample set was bracketed by a full set of calibration standards. Two standards were injected after every 10 samples. After 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 Inc., 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 sample standard deviation (SD) of niclosamide concentrations in the water and sediment. Linear mixed effects regression (LMER) analysis was used to predict the niclosamide concentrations in the water within the plots based on plot, depth in the water column, and time after application. An analysis of variance (ANOVA) table generated with the LMER fitted model was used to assess the variability of mean niclosamide concentrations based on plot, sample depth relative to the water column, and time post granular Bayluscide application. Water samples collected outside of the plots were analyzed using LMER analysis to predict niclosamide concentrations

based on plot, location relative to application plot, sample depth relative to the water column, and time after granular Bayluscide application. An ANOVA table generated with the LMER fitted model was used to assess the variability of mean niclosamide concentrations based on plot, location relative to application plot, sample depth relative to the water column, and time after granular Bayluscide application. Sediment samples were analyzed using LMER analysis to predict the niclosamide concentrations based on plot, and time after granular Bayluscide application. An ANOVA table generated with the LMER fitted model was used to assess the variability of mean niclosamide concentrations based on plot, and time after granular Bayluscide application.

The mass of niclosamide measured within the plots 15 minutes after application compared to the mass applied to the plot was estimated. The mean concentration in the water was multiplied by the mean volume of water above the plots to estimate the amount of niclosamide in the water column. The mean concentration of niclosamide in the top 4 cm of sediment was multiplied by the mean mass of sediment in the top 4 cm of the 500-$m^2$ plots to estimate the amount of niclosamide in the sediment. The amount of niclosamide in the water and sediment were added to give the amount measured in the plots.

## RESULTS

### *Water column*

Niclosamide concentrations in the water within all study plots (including all depths and times) were variable ($F=23.51_{(10, 690)}$, $P < 0.001$) and ranged from < 0.001 to 0.479 $mg \cdot L^{-1}$ (Table 4, Figure 5). The mean niclosamide concentration was 0.054 $mg \cdot L^{-1}$, SD = 0.068 $mg \cdot L^{-1}$. Concentration did not vary by depth ($F = 2.214_{(4, 690)}$; $P = 0.066$) but did vary by plot ($F = 18.656_{(5, 690)}$; $P < 0.001$) and time ($F = 132.967_{(1, 690)}$; $P < 0.001$). Niclosamide concentrations measured in water samples collected 10 meters outside the study plots were also variable ($F = 9.082_{(13, 585)}$, $P < 0.001$), ranging from < 0.001 to 0.530 $mg \cdot L^{-1}$ with a mean concentration of 0.032 $mg \cdot L^{-1}$, SD = 0.060 $mg \cdot L^{-1}$ (Figure 6). Similarly, niclosamide concentrations

outside of the plots did not vary by depth ($F = 0.153_{(4, 585)}$, $P = 0.962$) but did vary by plot ($F = 10.506_{(5,585)}$, $P < 0.001$), time ($F = 37.707_{(1, 585)}$, $P < 0.001$), and sampler location ($F = 9.073_{(3,585)}$, $P < 0.001$).

*Sediment*

Niclosamide concentrations in the sediment samples varied ($F = 13.76_{(11, 517)}$, $P < 0.001$) in the plots, ranging from < 0.001 to 30.730 $mg \cdot L^{-1}$ with a mean of 2.1 $mg \cdot kg^{-1}$, SD = 2.4 $mg \cdot kg^{-1}$, (Table 5, Figure 7). The niclosamide concentration varied across plots ($F = 13.016_{(5, 517)}$, $P < 0.001$) and time ($F = 44.984_{(1, 517)}$, $P < 0.001$). The mean niclosamide concentration in the sediment was more than an order of magnitude greater than the mean concentration in the water for the six plots.

*Niclosamide application recovery*

Approximately 134.66 g (57%) of the 236.25 g niclosamide applied to each study plot was measured 15 minutes after granular Bayluscide application. It was estimated that the water in the plots contained on average 44.89 g (19%) of the niclosamide originally applied, and the top 4 cm of sediment contained 89.78 g (38%). There were not enough water samplers outside the plots to make a good estimate of the amount of niclosamide that had dispersed out of the plots.

**DISCUSSION**

*Distribution in the water within the plot*

Niclosamide concentrations measured in the water column from 1 cm above the sediment-water interface to the surface were similar. This could be because lentic waters churn like large mechanical oscillators and water velocities of only a few mm/second can induce turbulent flow (Wetzel 1983). Surface waves create vertical cycloid movements below the surface water. These cycloid movements are able to transport a chemical from the sediment-water interface through the water column to the surface. The application boat's motor may churn and mix the water in a vertical fashion (Loberto 2007). In addition, any niclosamide adhering to the outside of the Bayluscide granules or exposed due to breakage of the

granules during application would likely start dissolving immediately on entering the water increasing the niclosamide concentration in the upper portion of the column. The niclosamide concentrations in the upper portion of the initial sampling are consistent with addition of unencapsulated niclosamide to the water column during application and warrant additional investigation. If niclosamide leaches off the outside of the encapsulated granules or if some granules are breaking open during application and exposing niclosamide early, then the full dose is not reaching the sediment. The concentrations that we observed in the water column were similar to those previously reported. The concentrations reflect the range of niclosamide solubility, which increases from 283 $\mu g \cdot L^{-1}$ at pH 7.0 to 34,800 $\mu g \cdot L^{-1}$ at pH 9.0 (Fathulla 1999) in the 7.4-8.5 pH water typically found in the Great Lakes basin (Bills et al. 2003). The water samples collected at hour 5 have a small rise in niclosamide concentration. These samples were collected midafternoon when the water pH is normally high and niclosamide is more soluble.

*Distribution in the water outside the plot*

While there were not enough water samplers deployed outside the plots to accurately calculate the amount of niclosamide, the presence of niclosamide outside the plots may warrant further investigation. Niclosamide concentrations at water samplers outside of the plots were similarly distributed vertically throughout the water column, generally decreased with time, and varied by location. The Left and Right and Lake sides had higher and more variable concentrations than the Land side. During treatments the application boat traveled across the plot then circled back around the plot via the Lake side to make the next pass through the plot. Granular Bayluscide was applied above the water in a sweeping motion off the front of the boat and chemical may have been applied outside of the plot or moved by currents produced by the boat propeller. Kayaks were used for sample collection and likely did not contribute to substantial water column disturbance. During typical surveys and treatments, plots are assessed with the application boats so there may be more mixing of the water column during assessment activities than what was experienced in this study.

*Distribution in the Sediment*

The niclosamide concentration in the top 4 cm of the sediment was generally more than an order of magnitude greater than in the water. The variability in the samples shows the distribution of the granular Bayluscide was not even across the sediment surface at the scale of our samplers which were 11 cm in diameter or roughly the length of a larvae. This variability likely obscured any trend when comparing niclosamide concentration to total organic carbon content.

*Efficacy Implications*

A lethal exposure is considered the chemical concentration an organism needs to be exposed to over a specific duration of time that will cause lethality. The amount of granular Bayluscide applied using U.S. Fish and Wildlife Service standard operating procedures would result in a niclosamide concentration of 9.275 mg/L in the bottom 5 cm of water above the substrate (Solomon 2019). This calculation assumes that all the niclosamide in the granular Bayluscide would completely dissolve only in the bottom 5 cm of the water column and would persist long enough to kill larvae before it dispersed throughout the entire water column or into the sediment. In our study the mean concentration in the water measured at 1 cm above the sediment was 0.066 $mg \cdot L^{-1}$, orders of magnitude lower than the calculated value. The mean sediment sample concentration was 2.1 $mg \cdot kg^{-1}$ $_{(wet\ wt)}$, which is roughly an order of magnitude greater than in the water layer above it. Niclosamide likely dissolves into the water and adsorbs to sediment at rates consistent with its limited water solubility (Fathulla 1999) and with the sediment's adsorption capacities (Dawson et al. 1986). Sediment may then act as a niclosamide reservoir. As the niclosamide in the water column is diluted/diffused, it is replaced by niclosamide that desorbs from the sediment. This is supported by Dawson et al. (1986) who reported niclosamide sediment desorption rates ranging from < 10% to > 60%, depending on organic content.

It is not known whether larvae obtain a lethal dose of niclosamide from granular Bayluscide through the column water, sediment pore water, or both. If exposure is from the water column only, it is unknown if

mortality may result from a long exposure at the low niclosamide concentrations found in our study. Rye and King (1976) reported a 24-hour lethal concentration to kill 50% of the test organisms (LC50) of 0.049 $mg \cdot L^{-1}$ niclosamide, and Scholefield and Seely (1992) reported a 9-hour LC50 of 0.052 $mg \cdot L^{-1}$ niclosamide for free swimming larvae. Dawson et al. (1977) reported 6-hour LC50s for free swimming larvae that ranged from 0.0389 – 0.0810 $mg \cdot L^{-1}$ niclosamide tested at 12 °C in water with pH values of 7.5 and 8.5, and an LC50 of 0.045 $mg \cdot L^{-1}$ niclosamide at 17 °C in water with a pH value of 7.5. They also reported a 12-hour LC50 (0.180 $mg \cdot L^{-1}$ niclosamide at 12 °C) for burrowed larvae that was 3 times greater than for free swimming larvae. Scholefield et al. (2003) determined regression lines for exposure times required to achieve 50 and 99.9% mortality of free swimming sea lamprey larvae in water baths (pH 7.8-8.3) with niclosamide ranging from 0.46 – 4.7 $mg \cdot L^{-1}$ at 12 and 17 °C. Using their regression lines the mean niclosamide concentration measured at 1 cm in our study (0.066 $mg \cdot L^{-1}$) would require an exposure time of 2.18 and 4.89 hours at 17 and 12 °C, respectively, to achieve 50% larval mortality and 3.40 hours and 7.94 hours at 17 and 12 °C, respectively, to achieve 99.9% larval mortality  These lethal exposures to free swimming larvae do not seem to align with observations that larvae burrowed in sand and exposed to granular Bayluscide have a mean emergence time of 37.3 minutes at 12 °C with 99% mortality in aquaria (Boogaard et al. 2016).

How niclosamide laden sediment and pore water contribute to toxicity is unknown. Previous toxicity studies used free swimming larvae or larvae burrowed in pure sand and niclosamide in the sediment was not measured. Larval sea lampreys mostly remain buried and are passive feeders, and they form a tube around the head and brachial region (bladder-like structure) to facilitate water current by gluing silt particles with a mucoid secretion of the endostyle and skin glands (Vladykov 1952). Moore and Beamish (1973) reported that sand and detritus were frequently found in the gut of larvae. It is not known whether the glued area would allow water in the sediment to move horizontally into the tube. If not, exposure via sediment may be a function of the degree to which sediment is pulled vertically into the tube from the sediment water interface. If niclosamide in the sediment is an important route of exposure, factors

contributing to uneven distribution of granular Bayluscide would affect mortality and treatment efficacy. Larvae located in areas with low amounts of granular Bayluscide may not receive a mortal dose. If it is found that the sediment is an important route of dose delivery, then a better understanding of the sediment surface area surrounding a burrowed larvae that needs to be treated to kill that larvae may be valuable information when trying to determine how fine of scale granular Bayluscide needs to be spread across the sediment. That in turn can guide the SLCP on whether modifications are warranted in their granular Bayluscide delivery systems.

In conclusion, we found that in lentic granular Bayluscide assessment plots the concentration of niclosamide in the water column is relatively low (mean = 0.054 $mg \cdot L^{-1}$) and dispersed fairly evenly throughout the water column. The niclosamide concentration in the sediment is relatively high (mean = 2.1 $mg \cdot kg^{-1}$) and not distributed evenly across the sediment surface.

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**ACKNOWLEDGEMENTS**

We thank Hanna Mello, Mary Henson, Mary Wilson, Stephen Lantz, Grace McCalla, and Bridget Ladell for their assistance with sample collection and or processing, Jean Adams and Richard Erickson for assistance with the statistical analysis and all the folks who have improved the report with their critical reviews. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

**DELIVERABLES**

*Reports*

Bernardy, J.A. Kaye, C.A., Schloesser, N.A., and Schueller, J.R. 2020. Distribution of Niclosamide Following Granular Bayer Applications in Lentic Environments. 30 pp.

*Data and Analysis Code*

Bernardy, J.A., Kaye, C., Schloesser, N.A., and Schueller, J.R. 2020. Distribution of Niclosamide Following Granular Bayer Applications in Lentic Environments data. U.S. Geological Survey data release. https://doi.org/10.5066/P9AO0E0N

*Presentations*

Bernardy, J.A. 2015. UMESC 2015 Progress. Presented at the Great Lakes Fishery Commission Lampricide Control Task Force meeting, Mackinaw City, Michigan, September 8, 2015.

Schloesser, N.A. 2014. Dissipation of Bayluscide in lentic areas. Presented at the Sea Lamprey Annual Working Session in Traverse City, Michigan, January 22, 2014.

**RESEARCH HIGHLIGHTS**

- Niclosamide concentration in water is fairly consistent vertically throughout the water column after a lentic granular Bayluscide application.

- The niclosamide concentration in the sediment was more than a magnitude greater than in the water column just above the sediment.

- The niclosamide concentration in the sediment and pore water was highly variable (<0.001 - 30.730 $mg \cdot kg^{-1}$) and likely the result of uneven distribution of the granular Bayluscide.

**Table 1.** Center coordinates of each 500-$m^2$ study plot used to assess the distribution of niclosamide after granular Bayluscide applications.

| Assessment Plots | Coordinates |
|---|---|
| ***Hog Island Creek*** | |
| **Plot A** | 46.07105N 85.28286W |
| **Plot B** | 46.07169N 85.28341W |
| **Plot C** | 46.07153N 85.28276W |
| **Plot D** | 46.07158N 85.28401W |
| **Plot E** | 46.07201N 85.28558W |
| ***Peshtigo River*** | |
| **Plot F** | 44.97441N 87.65385W |

**Table 2.** Water depth, temperature and pH measured at three water samplers within each study plot at Hog Island Creek (A-E) and Peshtigo River (F).

| Plot | Sampler | Depth (m) | Date | Time (hh:mm) | pH (si) | Temp (°C) |
|---|---|---|---|---|---|---|
| **A** | A | 0.88 | 6/5/2014 | 8:15 | 8.36 | 10.5 |
| | C | 0.94 | 6/5/2014 | 8:15 | 8.51 | 10.4 |
| | E | 1.01 | 6/5/2014 | 8:15 | 8.52 | 10.5 |
| **B** | A | 0.79 | 6/7/2014 | 8:15 | 8.50 | 14.2 |
| | C | 0.67 | 6/7/2014 | 8:15 | 8.51 | 14.2 |
| | E | 0.55 | 6/7/2014 | 8:15 | 8.53 | 14.2 |
| **C** | A | 0.53 | 6/9/2014 | 8:30 | 8.56 | 12.6 |
| | C | 0.61 | 6/9/2014 | 8:30 | 8.57 | 12.5 |
| | E | 0.67 | 6/9/2014 | 8:30 | 8.57 | 12.4 |
| **D** | A | 0.92 | 6/11/2014 | 7:56 | 8.31 | 12.3 |
| | C | 0.98 | 6/11/2014 | 7:57 | 8.32 | 12.3 |
| | E | 1.13 | 6/11/2014 | 7:58 | 8.33 | 12.3 |
| **E** | A | 0.66 | 7/2/2014 | 8:15 | 8.53 | 14.0 |
| | C | 0.71 | 7/2/2014 | 8:15 | 8.55 | 14.1 |
| | E | 0.68 | 7/2/2014 | 8:15 | 8.49 | 14.1 |
| **F** | A | 0.73 | 8/27/2014 | 8:45 | 7.95 | 22.1 |
| | C | 0.85 | 8/27/2014 | 8:45 | 7.95 | 22.1 |
| | E | 0.78 | 8/27/2014 | 8:45 | 7.95 | 22.1 |

**Table 3.** Total organic carbon content from a five-grab composite sediment sample per plot and temperature and pH of the first three control and last three treatment sediment samples collected at each plot.

| | | Control Samples | | | | Treatment Samples | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total Organic Carbon[a] | Date | Time | pH | Temp. | Date | Time | pH | Temp. |
| Plot | % | | hh:mm | si | °C | | hh:mm | si | °C |
| A | 0.140 | 06/04/14 | 17:50 | 8.53 | 18.1 | 06/05/14 | 16:24 | 7.61 | 18.3 |
| | | 06/04/14 | ≈17:50 | 8.42 | 18.4 | 06/05/14 | 16.22 | 7.69 | 18.5 |
| | | 06/04/14 | ≈17:50 | 8.44 | 18.1 | 06/05/14 | 16:34 | 8.17 | 17.0 |
| B | <0.017 | 06/06/14 | 12:51 | 8.58 | 14.8 | 06/07/14 | 16:10 | 8.28 | 19.3 |
| | | 06/06/14 | 12:51 | 8.55 | 14.8 | 06/07/14 | 16:13 | 8.01 | 19.2 |
| | | 06/06/14 | 12:54 | 8.54 | 14.7 | 06/07/14 | 16:06 | 8.02 | 19.2 |
| C | <0.017 | 06/08/14 | 12:04 | 8.58 | 14.4 | 06/09/14 | 16:13 | 8.28 | 18.0 |
| | | 06/08/14 | 12:06 | 8.32 | 14.8 | 06/09/14 | 16:10 | 8.41 | 17.7 |
| | | 06/08/14 | 12:07 | 8.48 | 14.7 | 06/09/14 | 16:00 | 8.71 | 18.1 |
| D | 0.350 | 06/10/14 | 11:04 | 7.26 | 15.3 | 06/11/14 | ≈ 15:40 | 7.13 | 15.9 |
| | | 06/10/14 | 11:06 | 7.4 | 14.2 | 06/11/14 | ≈ 15:40 | 7.37 | -[b] |
| | | 06/10/14 | 11:05 | 7.64 | 14.7 | 06/11/14 | ≈ 15:40 | 7.73 | 16.2 |
| E | 4.20 | 07/02/14 | ≈ 8:15 | 7.53 | 17.3 | 07/02/14 | 16:25 | 7.16 | 20.1 |
| | | 07/02/14 | ≈ 8:15 | 7.26 | 17.7 | 07/02/14 | 16:25 | 7.28 | 20.8 |
| | | 07/02/14 | ≈ 8:15 | 7.59 | 17.8 | 07/02/14 | 16:26 | 7.21 | 20.2 |
| F | 0.810 | 08/26/14 | 10:34 | 7.48 | 23.4 | 08/27/14 | 16:30 | 7.73 | 24.0 |
| | | 08/26/14 | 10:31 | 7.54 | 23.8 | 08/27/14 | 16:34 | 7.54 | 24.2 |
| | | 08/26/14 | 10:30 | 7.34 | 24 | 08/27/14 | 16:38 | 7.48 | 24.3 |

[a] Assay method MSA 29-3.5.2

[b] Temperature not recorded.

**Table 4.** Mean niclosamide concentration at five heights in the water column in six larval assessment plots after application of granular Bayluscide.

| Water Column Height | Niclosamide Concentration in Water $mg \cdot L^{-1}$ (SD) | | | | |
|---|---|---|---|---|---|
| | 15 minutes | Hour 1 | Hour 3 | Hour 5 | Hour 7 |
| 1 cm | 0.15 (0.13) | 0.077 (0.039) | 0.023 (0.013) | 0.046 (0.028) | 0.032 (0.024) |
| 13 cm | 0.11 (0.12) | 0.057 (0.035) | 0.019 (0.014) | 0.043 (0.029) | 0.027 (0.021) |
| 26 cm | 0.11 (0.12) | 0.056 (0.037) | 0.020 (0.014) | 0.041 (0.031) | 0.029 (0.021) |
| ½Height | 0.12 (0.13) | 0.059 (0.039) | 0.021 (0.014) | 0.042 (0.032) | 0.026 (0.017) |
| Surface | 0.096 (0.094) | 0.055 (0.046) | 0.021 (0.015) | 0.037 (0.025) | 0.021 (0.014) |
| Mean | 0.12 (0.12) | 0.061 (0.040) | 0.021 (0.014) | 0.042 (0.029) | 0.027 (0.020) |

**Table 5.** Mean niclosamide concentration in the sediment in six larval assessment plots after application of granular Bayluscide.

| Niclosamide Concentration in Sediment $mg \cdot kg^{-1}$ (SD) | | | | |
|---|---|---|---|---|
| 15 minutes | Hour 1 | Hour 3 | Hour 5 | Hour 7 |
| 2.9 (2.4) | 2.8 (3.7) | 1.9 (1.6) | 1.7 (1.9) | 1.3 (1.8) |

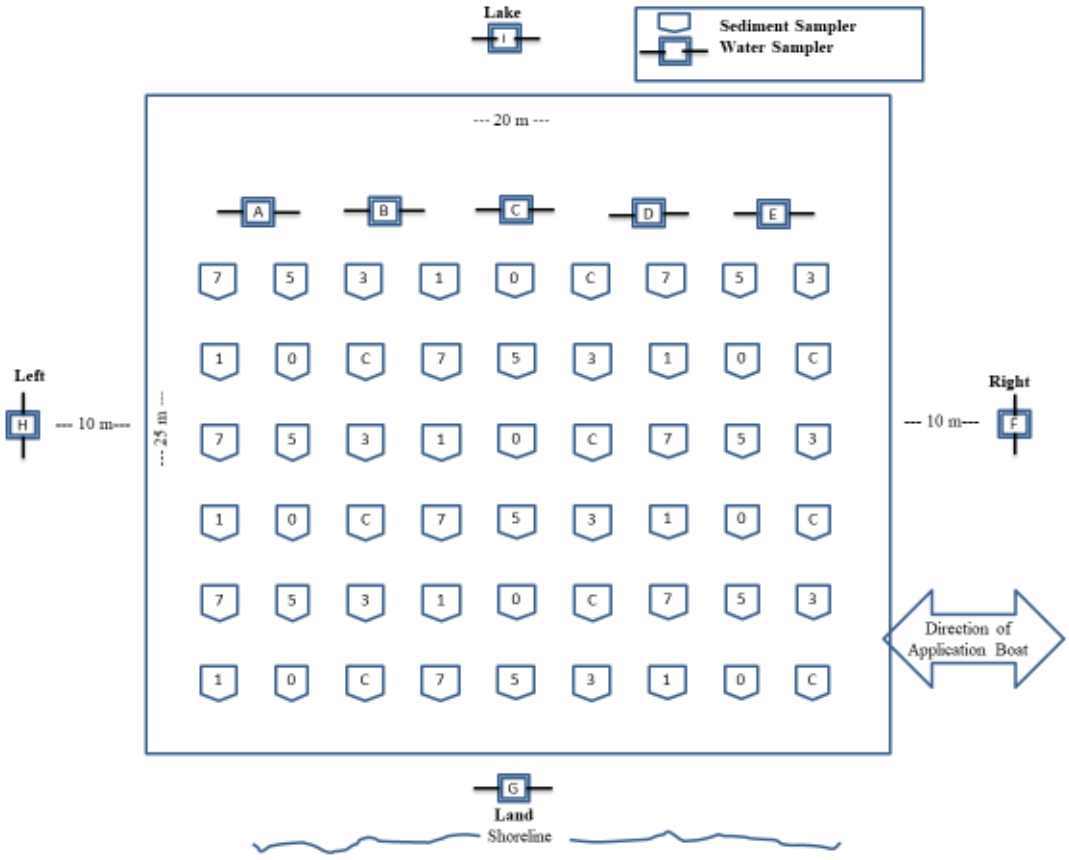


**Figure 1.** Water and sediment sampler layout in each granular Bayluscide assessment plot (drawing not to scale). The numbers designate the nominal hour the control (C) and test sediment samplers were retrieved after application.

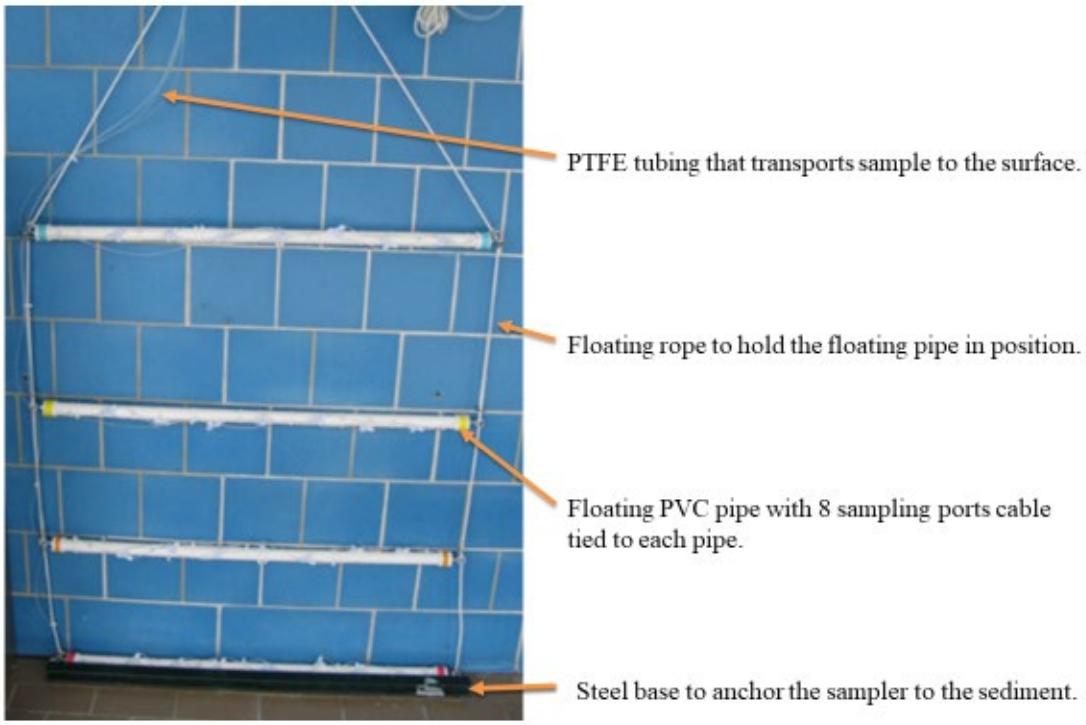


**Figure 2.** Sampler used to collect water from four heights in the water column inside and outside of the study plots.


**Figure 3.** Deployed sediment sampler in the bay at the mouth of Hog Island Creek (Mackinac County, Michigan). Each samplers was marked by a small labeled float fastened to lines used to lower and raise the sampler.

**Figure 4.** Sediment sampler: a tray (non-draining) with two non-draining 11-cm diameter x 4-cm deep dishes that were filled with sediment.

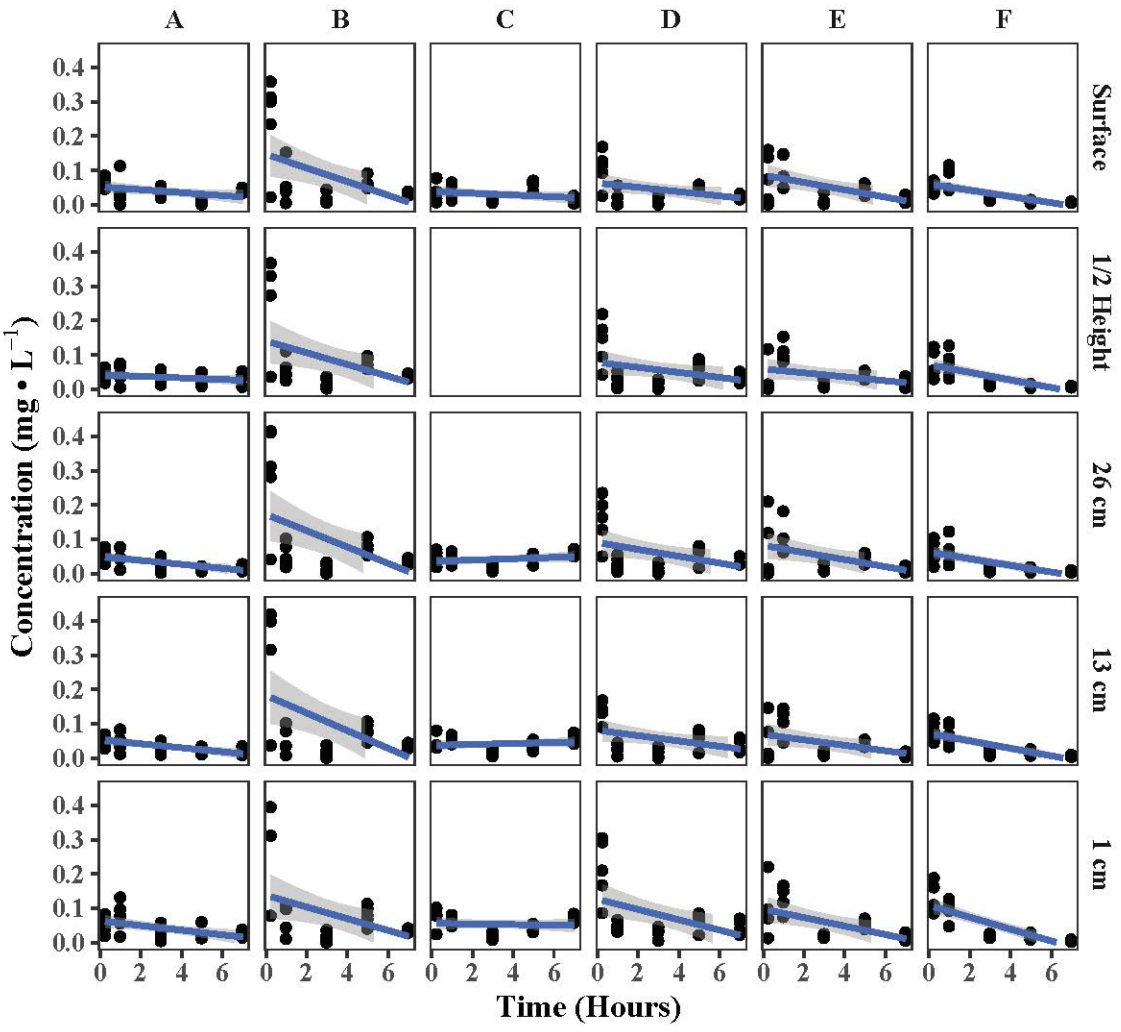


**Figure 5.** Concentration of niclosamide over time at five water column heights at five locations inside six (A–F) granular Bayluscide assessment plots. A linear model time trend with a 95% confidence interval (gray shading) is included for each plot.

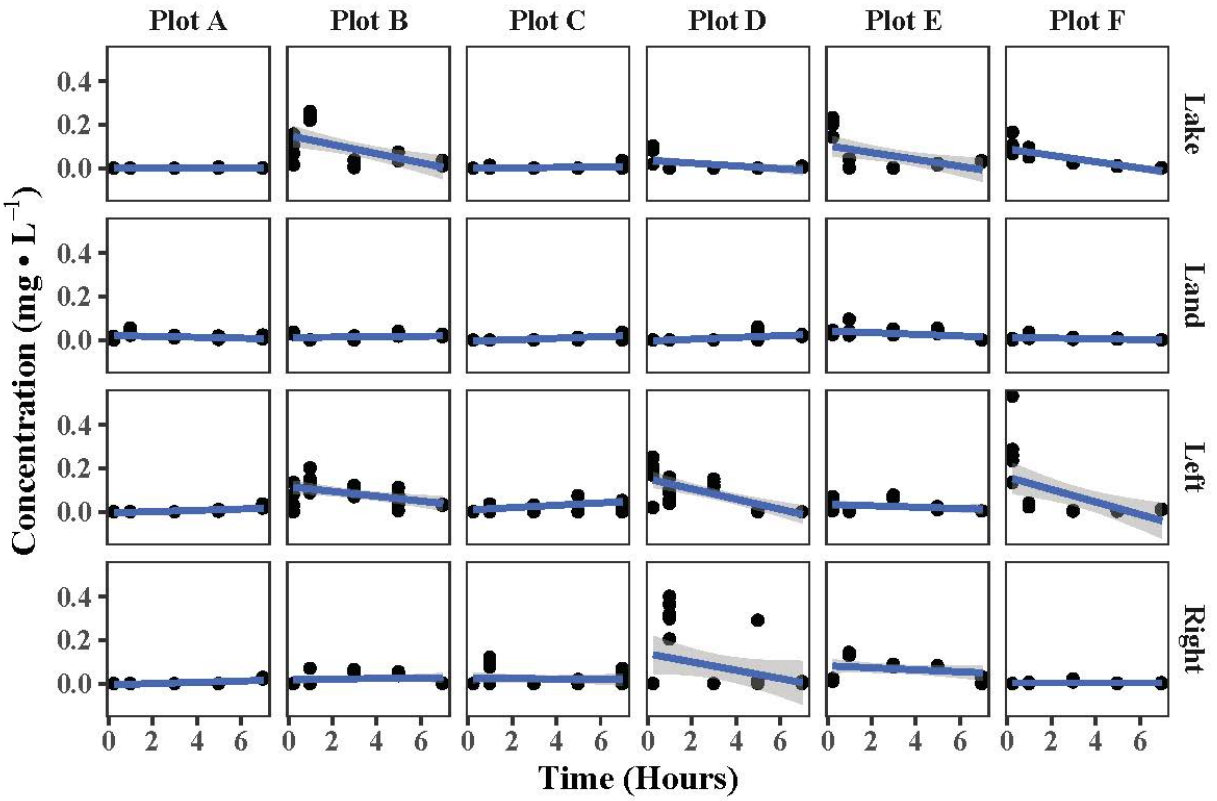


**Figure 6.** Concentration of niclosamide over time in the water column at four locations outside the six (A–F) granular Bayluscide assessment plots. A linear model time trend with a 95% confidence interval (gray shading) is included for each plot.

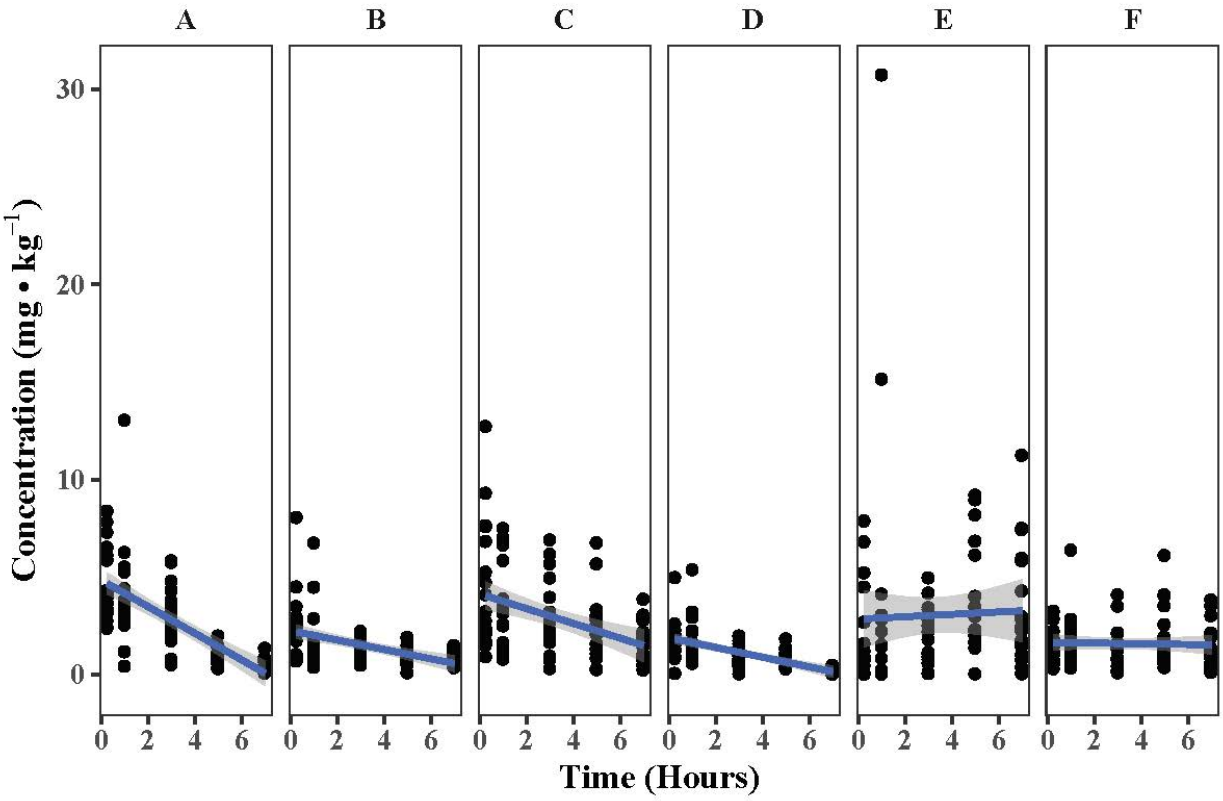


**Figure 7.** Concentration of niclosamide over time in sediment from six (A–F) granular Bayluscide assessment plots. A linear model time trend with a 95% confidence interval (gray shading) is included for each plot.