SCALING TO THE ORGANISM: AN INNOVATIVE MODEL OF DYNAMIC TOXIC HOTSPOTS IN STREAM SYSTEMS

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ABSTRACT

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Water quality modeling of lotic systems typically represents the distribution and transport of pollutants at the scale of the water body and is used in research and management. The distribution of various pollutants at the scale of the water body does not accurately represent the fine scale chemical exposure impacting benthic organisms. In flowing systems, fluctuations in the frequency, magnitude, and duration of exposure occur due to turbulence, therefore causing spatial and temporal variations in chemical exposure at the scale of the organism. Thus, a gap in knowledge exists in applying spatial models of toxicant movement at appropriate scales to predict exposure impacts on stream organisms. In order to characterize the fine scale distribution of pollutants in freshwater streams at the scale of a benthic organism, nine different artificial stream habitats were created (riffle, pool, run, bend, woody debris) with either sand or gravel substrate. Dopamine was released as a chemical tracer into the flume to mimic a groundwater source and measurements were recorded with a microelectrode and Epsilon electrochemical recording system. Ten sample points were taken throughout each habitat and recorded for five minutes. Peak length, peak height, and intermittency data were extracted and represented the frequency, magnitude, and duration of chemical exposure. Geographic Information Systems (GIS) and an Inverse Distance Weight (IDW) interpolation technique were used to spatially predict the chemical distribution throughout each of the habitats based on the measurements of ten sampling areas. Models were scaled to represent chemical distribution within and across habitats. Spatial and temporal variations of exposure were exhibited within and across habitats,
indicating that the frequency, magnitude, and duration of exposure is influenced by the organism’s location within a habitat and the habitat it resides in. The run and pool with sand substrate habitats contained the greatest frequency, magnitude, and duration of exposure, suggesting a more detrimental exposure compared to the other habitats. Conversely, no habitats measured in this study completely consisted of low frequency, magnitude, and duration of chemical exposure. The spatial and temporal fluctuations of fine scale exposure need to be considered in both ecotoxicology and water quality modeling to more accurately represent and understand the exposure of pollutants impacting benthic organisms.
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INTRODUCTION

Human impacts on the world’s freshwater supply are increasing at an alarming rate, leading to devastating consequences on the ecosystem (Camargo and Alonso 2006; Carpenter et al. 2011). Among the many harmful anthropogenic impacts, the release of pollutants poses a considerable concern. Every day, contaminants are released into the environment and enter our water through the hydrological cycle, as well as point and nonpoint sources (USEPA 2009). These toxicants are contaminating the Earth’s freshwater resources and causing health problems to humans that are exposed to severely polluted water. The neurophysiological effects of lead tainted water due to the Flint water crisis is a key example (Hanna-Attisha et al. 2016). However, not only are anthropogenic pollutants affecting humans, but organisms inhabiting the affected water body. Lead has been found to accumulate in fish, damaging their gills, livers, and kidneys (Palaniappan et al. 2008; Ahmed et al. 2014). Additionally, anthropogenic nutrient loading in Lake Erie can result in hypoxia (Arend et al. 2011) and eutrophication (Han et al. 2012), posing lethal and sub-lethal threats to organisms. Hypoxia has been found to stimulate ventilation in freshwater clams (*Corbicula fluminea*), increasing the uptake of heavy metals (Tran et al. 2008), while eutrophication has been found to alter feeding rates and predator-prey behaviors in perch (*Perca fluviatilis* L.) due to the resulting turbidity (Granqvist and Mattila 2004). In order to manage freshwater anthropogenic pollutants, many studies and agencies have relied on water quality modeling and simulations.

Water quality models allow us to understand and visualize pollutant fate and transport (Li and Duffy 2012; Li and Yao 2015), monitor the distribution of pollutants (Schwab et al. 2009), determine water quality and areas of concern (Nikolaidis et al. 2008; Gnanachandrasamy et al. 2015), and predict the exposure and consequences of pollutants on organisms (Burgert et al.
Numerous water quality models exist for lentic systems, specifically lakes (Schwab et al. 2009; Leon et al. 2011; Atkinson et al. 2012). Lake models often incorporate current velocities with concentration values recorded at various locations throughout the lake, being appropriate for a system at a large scale. The still nature of lakes offers less complex models when compared to models of lotic systems, such as rivers and streams.

Lotic systems are more complex and heterogeneous ecosystems than lentic systems because of its flowing water. Flow is a critical variable in ecotoxicology because chemicals are dispersed by advection (fluid flow) and diffusion through both air and water (Denny 1993). There are three different types of defined fluid flow that can regulate the movement of chemicals: laminar, turbulent, and transitional (transition from laminar to turbulent). Turbulence is an irregular and chaotic movement of eddies that create rapid mixing and dispersion (Vogel 1994; Sanford 1997), whereas laminar flow is more predictable and has less mixing (Davis 1986; Moore and Atema 1988; Vogel 1994). The spatial and temporal distribution of chemicals due to turbulence is known as a plume (Moore et al. 1994; Finelli et al. 1999). The distribution of such chemical plumes is not only determined by turbulence, but the interaction between fluid flow, substrate, and any obstacles that are present within a stream or river.

The heterogeneity and the hydrodynamics of rivers or streams depend greatly on the structural features within the stream and the interaction of the stream with the riparian zone (Frissell et al. 1986; Vidon and Hill 2004; Smiley and Dibble 2005; Thorp et al. 2013). One way to classify stream systems is through structural complexity or habitat type, including barriers (debris, wood, boulders, etc.), substrates and sediments (gravel/cobble, sand, transition), riffles, pools, and bends, which are basic features typical of freshwater streams (Frissell et al. 1986).
Smith et al. (1993) found that removing woody debris from a stream resulted in channel widening, a change in the primary flow path, bank erosion, and a change in the size of bars and pools. Flowing water creates a variety of habitats within a single river or stream, which results in different spatial and temporal distributions of anthropogenic chemicals (Thorp et al. 2006; Wolf et al. 2009; Edwards and Moore 2014). The current, velocity, and substrate of habitats in freshwater streams are important variables in the formation and variation of macroinvertebrate communities (Brunke et al. 2001; Gao et al. 2014).

The influence of habitat on the hydrodynamics and movement of chemicals in stream systems also influences macroinvertebrate exposure to pollutants (Pedersen and Friberg 2009; Rasmussen et al. 2012). Exposure is an important, but elusive concept when considering how toxicants are affecting the behavior, morphology, and physiology of the subjected organisms. Within classic ecotoxicological studies, exposure is defined and quantified by a predicted range of concentrations of a pollutant that alter the physiology or survivorship of test organisms. Under static conditions where exposure is defined by a set concentration over a certain period of time, studies fail to consider concentration fluctuations in natural systems due to turbulence (Moore et al. 1994; USEPA 2007; Gordon et al. 2012). Depending on the flow characteristics of the location of an organism, exposure may consist of varying “pulses” of chemical plumes contingent on the size of the turbulent eddies (Moore et al. 1994; Ludington and Moore 2017). The total average concentration of a toxicant within a stream will provide less information on an organism’s exposure than dynamic measurements of the frequency (number of pulses), magnitude (intensity of the pulse), and duration (time of the pulses) of the exposure of such chemicals (Diamond et al. 2005; Gordon et al. 2012). Therefore, measuring the mean concentration of a chemical in a system may not be sufficient when determining chemical
exposure at the scale of an organism (Handy 1994; Diamond et al. 2005; Ashauer et al. 2006; Zhao and Newman 2006; Gordon et al. 2012). Recent work has begun to elucidate the movement of chemicals through various freshwater habitats (Finelli 2000; Wolf et al. 2009; Edwards and Moore, 2014), as well as incorporating the impact of the episodic exposure of pollutants on freshwater organisms (Diamond et al. 2005; Butcher et al. 2006; Zhao and Newman 2006; Ludington and Moore 2017; Neal and Moore 2017).

While our understanding of the impacts of episodic exposure on organisms has expanded, ecotoxicological literature is missing a modeling component that incorporates both this dynamic concept of exposure, and measurements at the fine scale of an organism. Much of the current models revolve around modeling at the scale of the system itself. However, once toxicants enter a body of water, little is known about the fine scale movement of such toxicants throughout the water body or the watershed. The fine scale nature and the fluctuations of toxicant exposure are ecologically relevant to organisms as they respond to chemicals on the spatial scale of millimeters and temporal scale of seconds, or milliseconds (Finelli et al. 2000; Moore and Crimaldi 2004). Therefore, the concept of scale becomes important for both modeling and exposure (Dorigo et al. 2008). In order to quantify dynamic exposure at the scale of an organism, there is a need to incorporate the heterogeneity of fluid dynamics and stream geomorphology.

The purpose of this experiment was to determine and quantify fine scale exposure patterns throughout different habitats within a stream system and create spatial and temporal models representing the various aspects and distributions of exposure that is influencing benthic organisms. Aquatic ecotoxicology coupled with hydrodynamics and stream structure can provide valuable information to not only the understanding of exposure, but water quality modeling as well. The direct measurement of chemical movement in streams along with Geographic
Information Systems (GIS) and spatial interpolation modeling of the distribution of chemicals, can provide insight into the spatially and temporally dynamic nature of toxicant concentration and exposure. The identification and representation of such exposure patterns of pollutants in streams with commonly found physical features will allow us to further our understanding of ecotoxicology and hydrodynamics, and begin to use this information to improve the status of freshwater systems around the world.
MATERIALS & METHODS

Experimental Setup

An artificial stream, or flume (17.4 × 0.98 × 0.39 m: l × w × d), was built out of cinder blocks (40.6 × 20.3 × 20.3 cm) and 4 mil plastic sheeting at the Stream Research Facility at the University of Michigan Biological Station in Pellston, Michigan (45°33'50.1"N 84°45'04.2"W). The flume consisted of a mixing section (4.3 m) and a working section (3.0 m). The working section consisted of a sampling area (1.5 m), delivery system platform (0.3 m), and computer platform (1.2 m; Figs. 1, 2). The mixing section contained two collimators (spaced 1.78 m apart), each made from 3 pieces of plastic egg crating (1.7 cm² holes) and plastic screening in order to reduce turbulence and establish an equilibrium benthic boundary layer (Nowell and Jumars 1987; Moore and Grills 1999; Lahman and Moore 2015). The bottom of the flume was lined with either gravel (0.18 - 2.07 cm²) or sand substrate. Unfiltered water was pumped from the East Branch of the Maple River (Pellston, MI) and mixed with well water to create a water depth of approximately 22.9 cm. The flow velocity of the flume after the mixing section (6.8 ± 0.96 cm/s) was measured with a Hach Flow Meter (Hach FH950, Loveland, Colorado) at 4.5 cm above the substrate. Additionally, the flow velocity was measured for each of the stream habitats (described below) at 4.5 cm above the substrate. Water temperature was measured with a thermometer and ranged between 11 - 12°C. Flume outflow was pumped back into the Maple River and ensured the continual pumping of fresh water into the flume.

Stream Habitats

To create a simplistic, yet realistic foundation for our map, we chose physical features most often found in naturally flowing, freshwater streams. While streams vary in their characteristics, each of our physical features are typical for most constant flowing streams and
rivers (Frissell et al. 1986). For the purpose of our experiment, stream features containing unique flow regimes within a larger stream environment were considered to be a habitat. Measurements were taken from the following stream habitats:

- run
- woody debris
- riffle
- pool
- bend

Each habitat was located 9.14 m downstream of the inflow. The run, woody debris, pool, and bend sections were constructed in two different stream substrates (sand and gravel), whereas the riffle was constructed in only a gravel substrate, totaling nine different streams. The stream habitats were built and sampled in the 1.5 m sampling area of the flume. Samples were taken in ten different spatial locations throughout each of the habitats and remained the same between stream substrate types. For example, the sampling locations in the pool habitat with gravel substrate were the same as the pool habitat with sand substrate. However, the pool and the woody debris habitats had different sampling locations. The differences in structures between habitats made identical spatial sampling impossible. Additionally, the initial spatial location of the sampling points was chosen at random while ensuring that the points covered the majority of the area throughout the sampling area to produce better spatial fits for the model. Each of the sampling points were sampled at 4.5 cm above the substrate to measure at the scale and height of a larger macroinvertebrate.
Run

Smooth flowing runs are commonly found in natural streams. The run section (1.52 × 0.98 × 0.39 m) was replicated with both sand and gravel as the substrate, both measuring 1.5 – 2.0 cm deep (Fig. 3a). The flume flow rates in the gravel and sand run sections were 7.2 and 5.7 cm/s, respectively. The run segment provided a baseline for comparison of the other stream habitats sampled.

Woody Debris

The woody debris habitat (1.52 × 0.98 × 0.39 m) contained sunken branches from the East Branch of the Maple River randomly placed within the sampling area of the flume (Fig 3b). The logs ranged from 2.54 - 15.24 cm in diameter and 0.53 - 1.02 m in length. All ten sampling points were measured either 4.5 cm above the substrate or from the surface of the log, depending on the location of the points. The segment was recreated for both gravel and sand. The exact locations of the logs vary slightly between the sand and gravel substrates but the arrangement of all pieces remained the same to ensure that patterns could be compared between the two. The flow rate in front of the logs recorded 7.5 cm/s for both sand and gravel substrates.

Riffle

The riffle (0.61 × 0.98 × 0.36 m) was constructed of various sized rocks (5.68 – 532.26 cm$^2$) stacked randomly in a pile to imitate a shallow, rocky segment of a stream (Fig. 3c). Riffles are classified as having substrate sizes of 82.9 ± 56.3 mm (Cummins 1962; Jowett 1993). Therefore, the riffle was not replicated with a sand substrate. Additionally, several rocks were half above the water, breaking the surface of the artificial stream. A flow rate of 14 cm/s was measured 4.5 cm above the rocks in the center of the riffle. The sampling points were measured among the rocks (with the exception of a few points before and after the riffle).
Pool

The pool (1.52 × 0.98 × 0.39 m) had a flow rate of 0.8 cm/s for both sand and gravel substrates and was created between two riffles as previously described (Fig. 3a). The pool mimicked a deep segment of a stream, situated between two rocky, shallow segments. The entire pool was within the sampling area, while the two riffles were moved underneath the delivery system and computer platform (Fig. 1). All ten samples were measured in the pool segment.

Bend

The hydrodynamics in streams cause the outer banks to erode and deposit along the inner banks, creating meanders along the course of a stream (Thorne et al. 1985; Wang and Liu 2016). A meander consists of two sinuous bends, facing opposite directions. Our setup contained one bend, or one half of a meander, measuring 6.1 m long and 3.01 m wide (Fig. 3d). Sand and gravel gradually raised from 4 - 10 cm on the inside of the bend, while the remainder of the bend was 2 cm deep. Flume flow rate was 1.9 cm/s on the inner bank and 2.6 cm/s on the outer bank, for both sand and gravel substrates. The back end of the bend was sampled and situated within the sampling area of the flume (Fig. 2).

Delivery System

To imitate a groundwater point source, one 0.4 cm (inner diameter) vinyl Tygon tube was buried below the substrate approximately 0.18 m away from the delivery system platform and 0.48 m from the left wall of the flume. The tubing was attached to a reservoir bucket containing a stock solution of 1.13 g (per 10 liters of water) of a tracer element, dopamine. Water was continuously pumped into the 5-liter reservoir by a pump placed in a second 5-liter water reservoir to maintain the water level and a constant head pressure. Dopamine was released from the Tygon tubing at 60.9 ± 2.4 ml/min for all stream habitats and was mixed with fluorescein for
visual reference. Data collection began when the dye reached the end of the tubing. The Tygon tubing was placed beneath the substrate approximately 30 cm in front of the riffle as well as the woody debris.

Data Collection

An electrochemical detection system (Epsilon, Bioanalytical Systems, West Lafayette, Indiana) was used to measure the concentration of dopamine released as a chemical tracer. Attached to the Epsilon was an electrochemical recording microelectrode, constructed out of three 30 µm carbon fibers (1.3 - 1.9 cm in length), graphite-epoxy, 5-minute epoxy, and a copper wire with plastic coating (15.2 – 21.6 cm) contained within an outer glass pipette (3.8 – 5.1 cm; Fig. 4). The electrodes provide good spatial and temporal resolution (sampling rate up to 200 Hz), as well as specificity for biological stimuli (Moore et al. 1989; Wolf et al. 2009). A positive electrical charge is sent through the electrode and recorded by the Epsilon. The resulting electrical current is converted into concentration after initial calibration. A calibration curve was established by exposing the microelectrode to a series of three dopamine concentrations (1.0 mM, 2.0 mM, and 3.0 mM) to create a relationship between concentration and electrical current. This relationship was then used to convert data from current to concentration (Moore et al. 1989; Moore and Atema 1991). The microelectrode sampled at 20 Hz to provide a range in frequency that can be found in both turbulent systems and benthic organisms (Moore et al. 1989; Edwards and Moore 2014). In order to evaluate the chemical exposure that benthic organisms experience, the microelectrode was mounted to a tripod and placed 4.5 cm above the substrate to measure at the height at which a benthic organism would be found (Wolf et al. 2009; Edwards and Moore 2014). The microelectrode enabled the measurement of chemical concentration at temporal and
spatial scales for organisms (Moore et al. 1989) and generated data points for our model to visually represent chemical distribution.

Data Analysis

To quantify chemical dynamic characteristics, the concentration measurements were subdivided into different features that may be correlated with negative impacts of exposure such as magnitude, duration, and frequency (Ludington and Moore 2017; Neal and Moore 2017). Since Gaussian averages of chemical concentration are not what animals experience in flowing systems (Moore and Atema 1991; Finelli et al. 2000; Neal and Moore 2017), we evaluated chemical data collected at the relevant scale of a benthic organism. X-Y data (with x as time, and y as chemical concentration) were imported into an in-house excel program to extract temporal peaks or “pulses” of chemicals moving through the measured areas (Moore et al. 1989; Wolf et al. 2009; Edwards and Moore 2014; Lahman and Moore 2015). The peaks represent the concentration of the measured chemical at an instantaneous moment in time. The timing of these pulses is crucial to understanding benthic organism exposure and toxicity as this indicates how often organisms are exposed to fluctuating concentrations (Ashauer et al. 2006; Gordon et al. 2012; Ludington and Moore 2017; Neal and Moore 2017).

Successive peaks were subdivided into separate peaks if the concentration values decreased by 40% of the previous peak (Edwards and Moore 2014). Additionally, several features of a peak were calculated to provide insight on the movement and subsequent level of exposure within the stream. These parameters include, intermittency (time between each chemical peak), peak height (maximum concentration), and peak length (duration of peak and measure of exposure; Figs. 5a, 5b; Moore and Atema 1991; Moore et al. 1994; Wolf et al. 2009; Edwards and Moore 2014; Lahman and Moore 2015; Ludington and Moore 2017).
Intermittency, peak height, and peak length were targeted to capture components of the frequency, magnitude, and duration, respectively, of negative exposure.

**GIS Model Components**

Each of the peak parameters (length, intermittency, height, rise time, and absolute slope) were extracted from the in-house excel program for all ten sampling points in the nine different habitats. The peak parameter values (height, length, etc.) were averaged to obtain a single value per peak parameter, per sampling point. In order to scale the different spatial maps more effectively and to allow comparison, all data values underwent a logarithmic transformation.

The data were imported into ArcGIS 10.3.1 (Esri, Redlands, CA) to create spatial models demonstrating the chemical distribution throughout each of the stream habitats. Subsequently, our artificial flume had no coordinate system and in order to spatially model the chemical distribution in each habitat, a virtual Euclidian grid was placed over each habitat. From this grid, X-Y coordinates for each sampling location were measured for each of the ten sampling points per habitat and across all habitats. The bottom left corner of the sampling area in the straight flume (Fig. 1) and bend (Fig. 2) served as the origin (0, 0) coordinate. Both shapefiles accurately represent the sizes of the flumes and the locations of the sampling points.

Using an Inverse Distance Weight (IDW) technique for raster interpolation, we spatially predicted the grid values between each of our data points for each habitat. IDW takes the weighted sum of all the surrounding data points and averages them to determine the unknown sample points (ESRI 2002) and is a useful technique for modeling groundwater quality (Shomar et al. 2010; Malla et al. 2014; Gnanachandrasamy et al. 2015). The grid-like cells produced continuous fields that displayed a gradient in the peak parameter modeling chemical concentrations, which serve as representations of areas of increased and decreased exposure.
Using a red-blue color scheme, red was chosen for the highest values in peak height and length (as proxies for intensity and duration, respectively), and for the lowest values in intermittency (as a proxy for increased frequency of pulses) to represent more intense exposure, or areas most detrimental for organisms. Blue was chosen for the lowest values in peak height and length and the highest values in intermittency, representing less intense exposure, or areas least detrimental for organisms.

Scaling of Models

Log transformations were performed on all data points in order to standardize our models for comparisons. To create scales of the spatial models comparing different sites within a habitat, the color scale minimum and maximum values (for each particular peak parameter modeled) were selected from across the ten different sampling points (Table 1). Once the maximum and minimum values were determined, the scale was subdivided into ten equal intervals. Each of our within-habitat models were scaled to highlight the intensity of exposure, based on a location in a single habitat. To allow comparisons of spatial maps across different habitats, the minimum and maximum values for a particular peak parameter across all of the nine habitats were identified (Table 1). After these ends of the spectrum were found, the scale was subdivided into ten equal divisions. Our across-habitat models were scaled to characterize the intensity of exposure throughout several habitats commonly found in streams. We sought to determine which habitats were the most and least detrimental for benthic organisms throughout a stream system. Statistical analyses on the maps were performed by extracting pixel values from each of the across habitat models. A 2 × 4 model was constructed and evaluated using the Levene’s test to determine the homogeneity of variances between the models (R Development Core Team 2016; RStudio Team,
Boston, MA; Fox and Bouchet-Valat 2017). Since the riffle habitat was not replicated for sand substrate, it was not included in the analysis.
RESULTS

Comparisons Within a Habitat

Gravel Run

The three peak parameters modeled contained different spatial distributions within the run with gravel substrate (Fig. 6a). Longer peak lengths relative to the scale were distributed throughout the majority of the model, with the exception of short peak lengths upstream, in the left corner. Higher concentrations were located in the center of the flume and similar to the peak length measurements, an area of lower concentration was situated upstream in the left corner. Intermediate values were found downstream. The intermittency model was heterogeneous, displaying several long and short intermittency averages throughout the model (Table 1).

Sand Run

Peak length, height, and intermittency values were similarly distributed within the run habitat with sand (Fig. 6b; Table 1). Each model generally contained long peak lengths, higher concentrations, and short intermittencies relative to the model, and contained one major area of short peak lengths, low concentrations, and long intermittency averages in the same location (middle-right section of the model).

Gravel Woody Debris

Each of the peak parameters within the woody debris with gravel substrate habitat were heterogeneous and unevenly distributed. Several areas of long peak length averages relative to the scale were distributed downstream of the model and shorter areas of peak length averages were distributed in the middle (Fig. 7a). Mean peak height was more concentrated in areas near the input source, and less concentrated downstream. Mean intermittency was both short and long.
in the middle of the model and the remaining area had values that fell towards the mean (Table 1).

**Sand Woody Debris**

Our model of the woody debris with sand substrate habitat had a marked contrast for the upstream and downstream portions of the model (Fig. 7b). The mean values of peak length increased from upstream to downstream (Table 1). Both models for peak height and intermittency contained areas of higher concentrations and shorter intermittencies upstream and areas of lower concentrations and longer intermittencies downstream.

**Gravel Pool**

The peak length models within the pool with gravel substrate habitat contained areas of short peak lengths and were found at and behind the input source, as well as towards the downstream left corner of the model (Fig. 8a). Mean peak height contained areas of higher mean concentrations upstream of the model, on and behind the input source. Areas of lower mean concentrations were located downstream. The majority of the intermittency model displayed areas of long mean intermittencies, with areas of short mean intermittencies in the downstream left corner of the model and directly behind the input source (Table 1).

**Sand Pool**

Mean peak length within the pool with sand substrate models contained areas of long peak length averages found downstream, as well as small areas near the input source (Fig. 8b). Areas of shorter mean peak lengths relative to the scale were distributed within the center of the model as well as behind the input source. Mean peak height had less concentrated areas distributed throughout most of the model. Single areas of high concentrations were found both below and behind the input source. Mean intermittency contained intermediate values of the
range relative to the scale. Areas of longer average intermittencies were found downstream and in the middle, while areas of shorter intermittencies were found upstream, above the input source and in the middle of the stream (Table 1).

Riffle

The peak length and peak height models within the riffle habitat had similar spatial distributions (Fig. 9). Each model consisted of areas of longer mean peak lengths and higher mean concentrations throughout the entire model. A single area of short peak mean length and reduced concentration was located in the center of the model. Mean intermittency was heterogeneous, containing areas of long and short intermittency averages in the center of the model and areas of intermediate values relative to the scale throughout the rest of the area (Table 1).

Gravel Bend

Mean peak length within the gravel bend model) contained areas of short peak lengths upstream and in the middle portion of the model and areas of longer peak lengths at the end of the bend (Fig. 10a. A single area of high concentration relative to the scale was found in the center of the peak height model and less concentrated areas upstream and downstream. Areas in the intermittency models contained shorter intermittencies along the outer bank of the bend and longer intermittencies along the inner bank of the bend. The center of the model contained areas of both long and short intermittencies (Table 1).

Sand Bend

The bend with sand substrate habitat models consisted of areas of long peak lengths, higher concentrations, and short intermittencies (Fig. 10b). Each of the models contained a single area of short peak length, low concentration, and long intermittency on the inner bank, while
areas of long peak lengths, high peak heights, and short intermittencies were distributed among
the remaining area of the models (Table 1).

Temporal Comparisons

Time series moving models were created to visualize changes in peak length, peak
height, and intermittency over time. The models were broken into 30 second increments, where
peak parameters were analyzed separately for each 30 second increment and incorporated into
the temporally dynamic model. A higher variance resulted in greater fluctuations, while a lower
variance resulted in less fluctuations in the frequency, magnitude, and duration throughout the
span of the moving model (Table 2).

Comparisons Across Habitats

Peak Length

The riffle contained the longest peak length value (86.78 s) compared to any of the other
habitats. The woody debris habitat with sand substrate contained the second longest peak length
value (77.08 s). The run with gravel and sand substrate, the riffle, and bend with sand substrate
each contained a single area that contained no peaks (0 s). Surrounding the areas where no peaks
were detected, were areas of shorter peak length averages. The area of short average peaks in the
bend with sand substrate was located on the inner bank, where a buildup of deposited sand was
located. The bend with gravel substrate did not have an area of short peak lengths in the same
location. All of the models contained long peak lengths relative to the maximum and minimum
values found across all 9 habitats. Each habitat contained different variances and were found to
be heterogeneous (Table 2; Levene’s test, p < 2.2e-16).
Peak Height

The run with sand substrate contained the highest concentration value (4.64 µM) compared to all of the other habitats. The riffle contained the second highest concentration value (1.72 µM). Dopamine was not detected in areas within the run with both gravel and sand substrate, the riffle, and the bend with sand substrate. The run with sand substrate, riffle, pool with sand substrate, and the woody debris with gravel substrate were more concentrated habitats compared to the run with gravel substrate, pool with gravel substrate, woody debris with sand substrate, and the gravel and sand bends. The variances for each habitat were different from one another and were found to be heterogeneous (Table 2; Levene’s test, p < 2.2e-16).

Intermittency

The shortest area of intermittency was found in the pool with gravel substrate (2.12 s) and the second shortest was found in the bend with sand substrate (2.21 s). The longest average areas of intermittencies were found in the gravel run, sand run, riffle, and sand bend (300 s), as those areas contained no peaks. Each habitat contained both relatively long and short areas of intermittency to some degree. The run with gravel substrate and riffle contained more unevenly distributed areas of long and short intermittency than the remaining habitats. The run with sand substrate, pool with sand substrate, and both gravel and sand bends contained more areas of short intermittency, while the run with gravel substrate, riffle, and woody debris with sand contained more areas of long intermittency. All habitats were found to be heterogeneous and contained different variances (Table 2; Levene’s test, p < 2.2e-16).
The measurements recorded and models created in this study indicate three important findings for the field of ecotoxicology. First, at the scale of an organism, the distribution of chemicals is heterogeneous in space, therefore, organisms just a few centimeters apart may experience significantly different exposure conditions (i.e., concentrations, frequencies, or durations). Second, the exposure conditions of chemicals at a single point fluctuate greatly in time. Organisms at any location within a habitat will experience varying degrees of exposures to chemicals that may be orders of magnitude above any mean concentration. Average concentrations at a single point do not accurately describe the exposure that impacts an animal. Lastly, given the turbulent dynamics of different habitats, some habitats may have greater exposure and fluctuations of chemical concentrations than other habitats, even if the source concentration is the same. Consequently, some habitats may be more contaminated than others simply based on the interaction between the influx and flow environment and provide information about exposure.

This novel detection and visual representation of specific areas of exposure reveal the vast variation in chemical concentrations and exposure paradigms within and across habitats, and can help determine the most and least detrimental habitats and areas within a habitat for an organism. The representation of exposure in these models can be further explained as hot and cold spots. Areas greatest in magnitude, longest in duration, and highest in frequency relative to the data collected are considered hot spots of chemical exposure, indicative of harmful episodic exposure characteristics (Peterson et al. 2001; Gordon et al. 2012). Conversely, areas with the lowest magnitude, shortest duration, and lowest frequency relative to the data collected are cold spots of chemical exposure. Based on our models, these hot spot areas (red in color) can be
considered most detrimental to an organism, whereas the cold spots (blue in color) are least detrimental.

Spatial Heterogeneity Within Habitats

The heterogeneity of chemical distribution within a single habitat indicates that the location of an organism within a particular habitat is critical to its exposure. The run with gravel substrate contains, on average, long durations of chemical pulses, with lower magnitudes and shorter frequencies (Fig. 6a). Several areas of hot and cold spots are sporadically distributed throughout the habitat. Based on our run with gravel substrate models, a benthic invertebrate, for example, might experience a larger variance compared to other habitats for any of the exposure parameters, depending on the organism’s location within the habitat. Likewise, the hot spot in the bend with sand substrate contained peak lengths eight orders of magnitude longer, six orders of magnitude more intense, as well as two orders of magnitude more frequent compared to the cold spot (Fig. 10b; Table 1). Thus, benthic invertebrates located on top of the sand pile in the bend of a stream would be subjected to a much different level of exposure than one on the outer bank.

The heterogeneous distributions of such hot and cold spots within the various habitats are an important consideration for defining and quantifying chemical exposure. For example, the woody debris habitat with sand substrate provides a clear contrast between exposure in the up and downstream areas of a habitat (Fig. 7b). These exposures are driven by differences in flow characteristics which also drive habitat choice in benthic invertebrates (Hart and Finelli 1999; Larsson and Jonsson 2006; Johnson and Rice 2014). During the pupal stage, case-building caddisfly larvae attach their cases to solid objects such as logs. Caddisflies from the family Hydroptilidae are well represented throughout a number of stream habitats and flow regimes
The caddisflies situated in the upstream portion of the habitat would be exposed to short, intense, and frequent pulses of any anthropogenic toxicants introduced to the stream, whereas caddisflies of the same family downstream would be experiencing longer, but less intense, and less frequent pulses. The cold spots located in the downstream portion of the habitat would provide a refuge from frequent, high magnitude chemical pulses, whereas those organisms located directly upstream would not be provided with refuge of the same degree (Fig. 7b). Peak length and intermittency was one order of magnitude more intense and peak height was five orders of magnitude more intense in the upstream portion of the habitat. Thus, benthic invertebrates that make choices of their location within a habitat based off of flow characteristics are using the same characteristic (flow) that determines exposure at these scales (Edwards and Moore 2014; Ludington and Moore 2017).

**Temporal Heterogeneity**

The turbulent dispersion of toxicants results in a fluctuating distribution in time and space regardless of habitat (Wolf et al. 2009; Edwards and Moore 2014; Webster and Weissburg 2009; Ludington and Moore 2017; Neal and Moore 2017). The data extracted from the chemical pulses indicate that in addition to the spatial heterogeneity within and across habitats, there is heterogeneity within the temporal realm. Even at fine scales, our models represent only averages of exposure variables (peak height, peak length, intermittency) over our sampling period. Habitats exhibited both fluctuations and a higher variance than what is represented in our static models (Fig. 5a). This temporal variance within even a single location indicates a different style of exposure than our models represent. Indeed, work has shown that variance in the temporal aspect of exposure can alter the impact of a toxicant on an organism (Handy 1994; Diamond et al. 2005; Ashauer et al. 2006; Gordon et al. 2012; Ludington and Moore 2017; Neal and Moore...
Habitats may have hot and cold spots that are dictated by the degree of temporal fluctuations as well as the magnitude and variance of the fluctuation around a mean value (Fig. 5b).

The impact of temporal heterogeneity may be especially prevalent for sedentary or sessile organisms. For example, freshwater mussel larvae have been found to clump together, typically behind boulders in riffles and runs (Neves and Widlak 1987). A population of freshwater mussels in a riffle habitat, such as the one created for this study, would not experience the mean values of the quantified exposure parameters (5.50 s duration, 0.31 µM magnitude, 6.51 s frequency; Table 1), but rather the fluctuations of exposure over time (variance: 0.39 s duration, 0.22 µM magnitude, 0.02 s frequency; Table 1). Since these organisms are typically in the same location over sustained time scales, they are being exposed to the extreme highs and lows of exposure. The physiological, behavioral, and morphological impacts of toxicant exposure predicted by static exposure paradigms and models would not accurately predict organismal responses to such variable exposure patterns (Handy 1994; Diamond et al. 2005; Gordon et al. 2012; Neal and Moore 2017). These temporal effects may have greater implications on sedentary organisms than mobile organisms experiencing relatively average degrees of exposure, relative to the habitat and location.

Spatial Heterogeneity Across Habitats

Beyond spatial heterogeneity within a habitat and temporal heterogeneity within a single location, there is also spatial variation in exposure that occurs across different habitats. The flow characteristic of a specific habitat will determine the degree and magnitude of fluctuations in toxicant concentration that will impact organisms located in those habitats (Wolf et al. 2009; Edwards and Moore 2014; Lahman and Moore 2015; Ludington and Moore 2017; Neal and
Moore 2017). Given that each of the habitats measured in this study have different flow patterns, each of these habitats will also have unique variations in toxicant concentrations (and hence, exposure patterns). For example, riffle-pool sequences often occur in streams, each containing unique communities of organisms, as well as organisms that inhabit both riffs and pools (Logan and Brooker 1983; Brown and Brussock 1991; Lancaster et al. 2010). Organisms such as stonefly nymphs thrive in riffs because of the high water velocity and oxygen availability (Genkai-Kato et al. 2005), whereas organisms such as the big claw crayfish (*Orconectes placidus*) prefer the slower water velocity and depth of pools (Bishop et al. 2008). Therefore, despite what could be similar source concentrations of toxicants, these two habitats will contain orders of magnitude differences not only in mean values of exposure parameters, but also in the variance of those parameters around the mean. As a consequence, habitat choice (riffs and pools) influences standard ecological parameters such as predation risk and resources (Worischka et al. 2012; Clark et al. 2013), as well as exposure patterns.

Based on our models, an organism, such as a mayfly, which inhabit both riffs and pools (Finlay et al. 2002), would, on average be exposed to chemical pulses of long duration, high magnitude, and low frequency in a riffle. However, if the same organism moved downstream to a pool with gravel substrate, the organism would be exposed to pulses on average of long duration, low magnitude, and low frequency (Fig. 8a). Aquatic organisms tend to be more abundant in riffs in lower order streams as opposed to pools (Rabeni and Minshall 1977; Brown and Brussock 1991), yet may be subjected to a more detrimental exposure. In contrast, if the mayfly moved downstream to pool with sand substrate, the less detrimental habitat would therefore be the riffle since pools with sand substrate contained long durations, high magnitude, and high frequency of chemical pulses (Fig. 8b).
Effects of Flow

The distribution of pollutants within and across habitats are spatially and temporally heterogeneous due to flow. Flow determines not only the channel morphology of streams, but ecological processes such as dispersal, habitat use, predator-prey interactions, resource acquisition, competition, and sensory capabilities for benthic organisms (Moore et al. 1994; Hart and Finelli 1999; Moore and Grills 1999; Meissner et al. 2009). Variations in the morphology of a lotic system, as well as substrate and habitat type due to flow alters the ecological processes of benthic organisms and the degree of exposure depending on their location within a system (Edwards and Moore 2014; Ludington and Moore 2017).

Variations in substrate and habitat type of aquatic ecosystems therefore indicate flow patterns and exposure paradigms. Turbulent systems generate eddies and involve the chaotic movement of water, and thus chemical plumes (Sandford 1997). The more turbulence within a system, the greater the fluctuations in the frequency, magnitude, and duration of chemical pulses (Moore et al. 1994; Wolf et al. 2009). An increase in turbulence results in an increase of plume dispersal and number and frequency of plume patches (Moore et al. 1994). Additionally, roughness elements in the flow (obstructions or substrate such as gravel or sand) alter flow and the levels of turbulence in a system (Mutz 2000; Crowder and Diplas 2002; Jackson et al. 2007; Wolf et al. 2009; Edwards and Moore 2014; Ludington and Moore 2017). Any structure that alters flow will also influence the degree and fluctuations of exposure to toxicants independent of their mode of introduction into a habitat (Ludington and Moore 2017). Therefore, habitats with larger roughness Reynolds numbers have greater fluctuations in exposure (Moore et al. 1994; Wolf et al. 2009; Edwards and Moore 2014). In contrast, habitats with less mixing and lower levels of turbulence are less chaotic and have lower fluctuations in exposure. Decreased
fluctuations have implications of a more detrimental exposure as the flow is not altered as much and is more systematic (Handy 1994; Diamond et al. 2005; Gordon et al. 2012; Edwards and Moore 2014).

**Management and Monitoring Implications**

Water quality modeling of pollutants is a useful tool for answering a number of questions involving spatial and temporal distribution patterns, transport, concentrations, and management of the contaminants (Lam and Fohrer 2012; Trolle et al. 2014). Often, these questions are related to the exposure of organisms, including humans, and regulations are based off of the findings (Jiang et al. 2016; Gnanachandrasamy et al. 2015; Nikolaidis et al. 2008). However, it is important to consider the scale of the question at hand. Large scale modeling of flowing water bodies (rivers, streams) are not delineated at a scale small enough to accurately depict the degree of exposure organisms are experiencing, particularly benthic organisms. Our models incorporate a scale relevant to exposure for organisms and are an important step to bridging ecotoxicology and modeling.

Our models can be utilized in determining the degree of exposure organisms may experience in any stream or river habitat similar to the habitats we modeled. Our findings are beneficial to not only our understanding of chemical movement, but in determining which areas of a stream need to be focused on for rehabilitation and mitigation efforts. High magnitude, long duration, and high frequency exposure is considered to be most harmful to organisms as those parameters are indicative of a high degree of exposure (Peterson et al. 2001; Gordon et al. 2012). All of these characteristics were found in both the run and pool based habitat with sand substrate. Therefore, these two types of areas could be especially detrimental to animals subjected to chemical exposure based on our findings. There were no habitats containing low peak heights,
short peak lengths, and long intermittencies, which would be considered ideal exposure conditions for an organism. Because there is not enough knowledge of the specific morphological, physiological, and behavioral effects of varying degrees of peak height, peak length, and intermittency individually, a gap in knowledge exists in determining the combinations of the three parameters that provide a spectrum of impairment between the two ends of most and least detrimental exposure.

Currently, efforts to reduce the prevalence of toxicants in freshwater systems focus on surface water or random sampling throughout the water column. However, the impacts of such toxicants at the scale of an organism, as well as habitat specific distributions are not considered. The chemical hot spots found in the different stream features can be used to assess the degree of toxicity animals typical of that area are experiencing. This knowledge will allow us to better understand the effects of toxicants on organisms, as well as pinpoint problem areas to eliminate the pollution from the input source. In addition to understanding the linkage between habitat and organism exposure, surface flow types have been found to predict the hydraulic conditions and substrate type of stream habitats, as well as macroinvertebrate distributions (Reid and Thoms 2008). Using this information, coupled with the information gained from our models, we can predict the degree of exposure organisms will experience and combine that with large scale models. The combination of hydrodynamics and aquatic ecotoxicology will allow us to narrow conservation efforts and increase productivity of these efforts.

Conclusions and Future Directions

The spatial and temporal heterogeneity of the frequency, magnitude, and duration of chemical movement in flowing systems implies that exposure is not constant or static. However, many studies in ecotoxicology measure the concentration of a pollutant in a single point in space
and time (i.e. LC50 studies). Static exposure measurements do not capture the heterogeneity of exposure and the behavioral, morphological, and physiological effects of this heterogeneity on organisms (Handy 1994; Brent and Herricks 1998; Butcher et al. 2006; Zhao and Newman 2006; Gordon et al. 2012). These measurements are missing the episodic exposure component and the fluctuations in frequency, magnitude, and duration found in natural systems; however, studies have begun to incorporate such ideas to ecotoxicological research (Edwards and Moore 2014; Lahman and Moore 2015; Ludington and Moore 2017; Neal and Moore 2017).

Our models represent a new direction in ecotoxicology of redefining and completing the definition of exposure. The spatially and temporally heterogeneous nature of exposure (frequency, magnitude, duration) within and across habitats is an aspect that must be considered when exploring the effects of anthropogenic chemicals. There is a need for developing a combination of the components of exposure (peak length, peak height, and intermittency) into a single model to represent a complete exposure paradigm, or an equation of exposure. Work has begun to parse out the variations of different parameters of toxicant pulses and their effects on aquatic organisms under various levels of turbulence (Ludington and Moore 2017). Additionally, there is a need to build upon the models created in this study representing exposure relevant in nature and at scales relevant to organisms at various scales and systems. Enhancing our understanding of exposure becomes even more useful if we understand exposure at various scales within a system (benthic, middle, surface), as well as across systems (lotic, lentic) at a larger scale.


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<table>
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<tr>
<th>Within Habitat</th>
<th>Peak Length (s)</th>
<th>Peak Height (µM)</th>
<th>Intermittency (s)</th>
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<td>Range</td>
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<td>Var.</td>
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Table 1. Ranges, means, and variances (Var.) in peak length, peak height, and intermittency pixel values for each of the within-habitat models. Maximum and minimum values represent the high and low values relative to individual or collective habitats and were used to scale the within-habitat models. Values below are not log transformed.
<table>
<thead>
<tr>
<th>Habitat</th>
<th>Peak Length (s)</th>
<th>Peak Height (µM)</th>
<th>Intermittency (s)</th>
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Table 2. Variance values for each habitat for peak length, peak height, and intermittency obtained from the across-habitat models, calculated using Levene’s test. The riffle* was not included in the analysis because it was not replicated for sand substrate.
Figure 1. Top-down diagram of the flume built at the UMBS stream laboratory in Pellston, Michigan. Shaded black arrows indicate the direction of flow. Figure not drawn to scale.
Figure 2. Top-down diagram of the flume with the bend built at the UMBS stream laboratory in Pellston, Michigan. Bend width is 0.98 m and bend radius is 2.79 m. Shaded black arrows indicate the direction of flow. Figure not drawn to scale.
Figure 3. Top down diagrams of each stream segment. Each segment was replicated for gravel and sand (except for Fig. 3c). Diagrams include run and pool (3a), woody debris (3b), riffle (3c), and bend (3d).
Figure 4. Diagram of electrode used for data collection. Figure not drawn to scale.
Figure 5. Dopamine (µM) tracer plumes over time (s). Graphical representation of intermittency (time between each chemical peak), peak height (maximum concentration), and peak length (duration of peak and measure of exposure).
Figure 6. Spatial models of the run with gravel substrate (6a) and the run with sand substrate (6b) modeling log transformed peak length, height, and intermittency (left-right). Each of the within-habitat models were scaled to highlight the intensity of exposure within a single habitat. Water flows from top to bottom.
Figure 7. Spatial models of the woody debris with gravel substrate (7a) and the woody debris with sand substrate (7b) modeling log transformed peak length, height, and intermittency (left-right). These models are scaled to highlight the intensity of exposure within an individual habitat. Water flows from top to bottom.
Figure 8. Spatial models of the pool with gravel substrate (8a) and the pool with sand substrate (8b) modeling log transformed peak length, height, and intermittency (left-right). These models are scaled to highlight the intensity of exposure within a single habitat. Water flows from top to bottom.
Figure 9. Spatial models of the riffle modeling log transformed peak length, height, and intermittency (left-right). These models are scaled to highlight the intensity of exposure within a single habitat. Water flows from top to bottom.
Figure 10. Spatial models of the bend with gravel substrate (10a) and the bend with sand substrate (10b) modeling log transformed peak length, height, and intermittency (left-right). These models are scaled to highlight the intensity of exposure within an individual habitat. Water flows from top to bottom.
Figure 11. Spatial models of the various habitats modeling log transformed peak length. These models are scaled to highlight the intensity of exposure across habitats. Water flows from left to right and models were rotated to maximize use of space.
Figure 12. Spatial models of the various habitats modeling log transformed peak height. These models are scaled to highlight the intensity of exposure across habitats. Water flows from left to right and models were rotated to maximize use of space.
Figure 13. Spatial models of the various habitats modeling log transformed intermittency. These models are scaled to highlight the intensity of exposure across habitats. Water flows from left to right and models were rotated to maximize use of space.