

**Water Resources Research Center
Annual Technical Report
FY 2007**

Introduction

This report covers the period March 1, 2007 to February 28, 2008, the 42nd year of the Massachusetts Water Resources Research Center (WRRC). The Center is under the direction of Dr. Paula Rees, who holds a joint appointment as Director of the WRRC and as Director of Education and Outreach for the Center for Collaborative Adaptive Sensing of the Atmosphere at the University of Massachusetts Amherst. Dr. Stephen Mabee of the UMass Amherst Department of Geosciences continued work on a three-year 104G USGS grant to look at A Regional Approach to Conceptualizing Fractured-Rock Aquifer Systems for Groundwater Management. This is the fourth year of the study, and a no-cost extension was granted to continue the research until December 2008.

At the University of Massachusetts Amherst, Dr. Baoshan of the Plant, Soil, and Insect Sciences Department started a two-year project on the Environmental Behaviors of Engineered Nanoparticles in Water.

Piotr Parasiewicz of Mount Holyoke College concluded his research Using Hydromorphological Signatures to Determine Flow Related Habitat Thresholds for Instream Communities.

Finally, a graduate student grant was awarded to Lauren Moffat of the University of Massachusetts Amherst Animal Biotechnology and Biomedical Sciences Department to study Development of a standardized protocol for fish bioassays detecting estrogenic exposure.

Other projects conducted at WRRC include the Acid Rain Monitoring Project, the Massachusetts Stormwater Technology Evaluation Project, and continued collaboration with UMass Extension on a stream continuity project.

The fourth annual water resources research conference, Sustainable Waters in a Changing World: Research to Practice, was held at UMass on April 9, 2007, and planning took place for the fifth annual conference, Integrating Water Resources Management.

Research Program Introduction

None.

A Regional Approach to Conceptualizing Fractured–Rock Aquifer Systems for Groundwater Management

Basic Information

Title:	A Regional Approach to Conceptualizing Fractured–Rock Aquifer Systems for Groundwater Management
Project Number:	2003MA19G
Start Date:	9/30/2003
End Date:	12/27/2008
Funding Source:	104G
Congressional District:	1st District of MA
Research Category:	Ground–water Flow and Transport
Focus Category:	Water Supply, Groundwater, Water Quantity
Descriptors:	fracture characterization, domain analysis, well yield, fractured rock aquifers, groundwater availability, groundwater mapping, borehole geophysics
Principal Investigators:	Stephen B. Mabee, Michele Cooke

Publication

1. Manda, A.K; S.B Mabee, D.U. Wise, 2008, Influence of rock fabric on fracture attribute distribution and implications for groundwater flow in the Nashoba Terrane, Eastern Massachusetts, *Journal of Structural Geology*, (30) 464–477.
2. Manda, A.K, S.B. Mabee and D.F. Boutt, 2006. Characterizing fractured crystalline bedrock aquifers using hydrostructural domains in the Nashoba terrane, eastern Massachusetts. *Geological Society of America Annual Meeting, Philadelphia, Abstracts with Programs*, v.38, no.7, p.25.
3. Diggins, J.P., D.F. Boutt, A.K. Manda and S.B. Mabee, 2006. Estimating bulk permeability of fractured rock aquifers using detailed outcrop data and discrete fracture network modeling. *Geological Society of America Annual Meeting, Philadelphia, Abstracts with Programs*, v.38, no.7, p.223.
4. Boutt, D.F., A.K. Manda, S.B Mabee, J.P. Diggins, 2006, Characterizing fractured crystalline bedrock aquifers using discrete fracture networks in the Nashoba Terrane, Eastern Massachusetts, *Eos Transactions, American Geophysical Union*, v. 87, no. 52, Fall Meeting Supplement, Abstract H13D–1429.
5. Manda, A.K., S.B. Mabee and S.A. Hubb., 2005. Field mapping and fracture characterization techniques predict groundwater preferential flow paths in fractured bedrock aquifers, Nashoba terrane, MA. *EOS Transactions, American Geophysical Union*, v.86, no. 52, Fall Meeting Supplement, Abstract H23E–1477.
6. Manda, Alex K., Stephen B. Mabee, 2008 (In prep) Contrasting various fracture sampling methods from layered rocks, Submitted to *Hydrogeology Journal*.
7. Manda, Alex K., Stephen B. Mabee, David F. Boutt, 2007, Discrete fracture network modeling of hydrostructural domains: an example from Eastern Massachusetts, “in” 2007 US EPA/NGWA Fractured rock Conference: State of the science and measuring success in remediation, September 24–26, 2007, Portland Maine: national Groundwater Association (CD–ROM).

Introduction

This report presents the current status of the project with specific emphasis on significant progress and findings made to date. In the past year, a study documenting the spatial and statistical distributions of fractures in the Nashoba Terrane was published in the *Journal of Structural Geology* (Manda et al. 2008). This study documents how fracture characterization techniques were used to assess the distributions of fractures with respect to location, rock type and fracture type (e.g. joints and foliation parallel fractures) in the Nashoba Terrane. In addition, numerical modeling experiments were conducted to assess the relative importance of joints and foliation parallel fractures in influencing groundwater flow in the fractured crystalline terrane.

After numerical simulations of groundwater flow were conducted in the synthetic fracture networks, the question of how well synthetic fracture networks matched natural systems arose. In question was whether the fracture sampling technique that was used in the Nashoba terrane was robust enough to capture the statistical and spatial distribution of fractures within a given structural domain. To address this question, another numerical modeling experiment was devised to evaluate three of the most common fracture sampling techniques (Manda and Mabee, submitted to *Hydrogeology Journal*). Employing a combination of Geographic Information Systems (GIS) and discrete fracture network (DFN) modeling techniques, an image of fractured and layered Silurian dolomites was digitized, from which fracture attributes were collected and used to build DFN models. The single scanline, multiple scanline, and selection methods were used to collect fracture data from the digitized fracture network in a GIS environment. Porosity and potential permeability results from models created using data from the three sampling techniques were then used to evaluate the effectiveness of each fracture sampling technique.

Numerical simulations of groundwater flow are currently being used to investigate the role that fracture characteristics (i.e. trace length, number of fracture sets, angle of intersection between fracture sets) play in transmitting and storing fluids in fractured crystalline rocks. In recent studies (e.g. Surette and Allen, 2008; Surette et al. 2007), packages of rocks with similar hydraulic properties have been delineated based on the hydrostructural domain concept (Mackie, 2002). According to this concept, hydraulic properties in rock volumes vary as a direct consequence of differences in fracture intensities of the rock volumes. However, preliminary analyses conducted indicate that other than intensity, other fracture characteristics affect the transmissive and storage properties of fractured media. Hence, it appears that the definition of the hydrostructural domain ought to be expanded to include fracture attributes such as trace length and angle of intersection.

Fracture Attribute Distribution in the Nashoba Terrane

An in-depth description of this section can be found in Manda et al. (2008). Over 3000 fracture measurements were collected from 78 outcrops in the multiply deformed crystalline terrane of the Nashoba Terrane (Fig. 1). For this study, the different rocks were subdivided into the Nashoba Formation, Marlboro Formation, Andover Granite, Sharpners Pond Diorite and Newbury Volcanics. Fracture types collected in the terrane include steep joints, sheeting joints, foliation parallel fractures and veins (Fig. 2). Two prominent NE-SW and NW-SE trending fracture sets are observed in the terrane. Further, the trends of the veins and the foliation parallel fractures are subparallel to the NE-SW trending joints set. However, on closer inspection, the trend of the foliation parallel fractures is not consistently NE-SW but rather rotates from $\sim 035^\circ$ in the southwest of the terrane to ~ 070 in the northeast. It appears the foliation and foliation parallel fractures subparallel the Nashoba terrane axis (Fig. 3).

The influence of lithology and fabric on fracture trace length and spacing was evaluated for both steep joints and foliation parallel fractures. Fracture spacing and trace length frequency distributions of foliation parallel fractures and joints are lognormal (e.g. Fig. 4). However, foliation parallel fractures are more closely spaced than joints. Foliation parallel fractures have a median trace length of ~ 0.15 m, half that of steep joints. Fracture spacings for foliation parallel fractures are controlled by the rock fabric, where the planes of penetrative fabric are closely spaced. These planes act as weaknesses along which

new fractures (i.e. foliation parallel fractures) ultimately develop when the rocks are subjected to favorable stress magnitudes and favorably oriented stress fields.

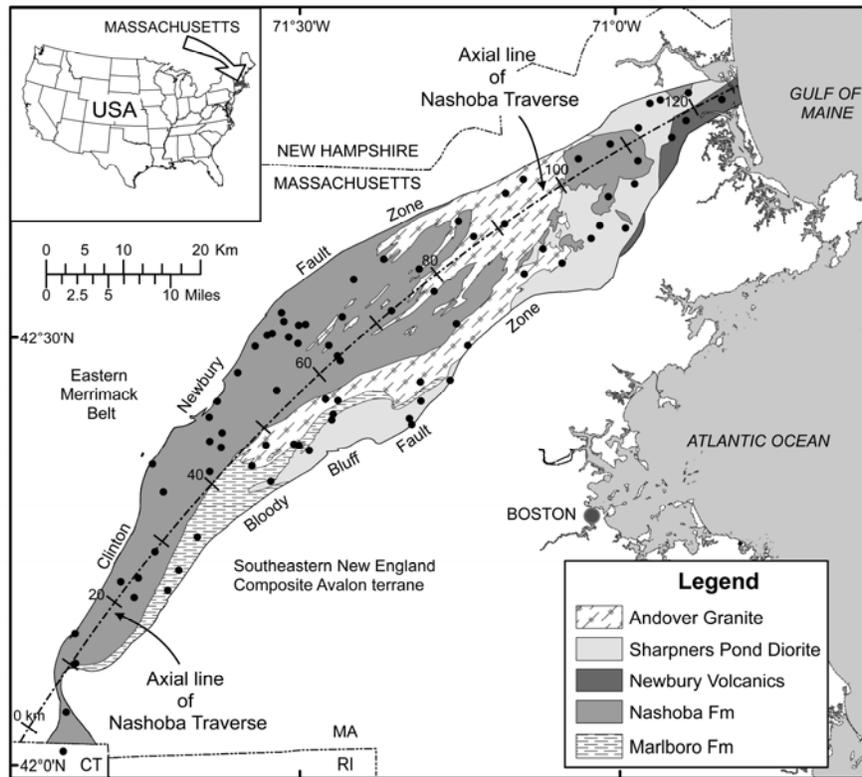


Figure 1. Simplified bedrock geologic map of the fault bounded Nashoba terrane located in eastern Massachusetts. Stations with fracture data are shown as black circles. Rock groups on the map are based on major rock units from the bedrock geologic map of Massachusetts (Zen et al., 1983). Dashed line represents the Nashoba axial traverse from which fracture data in 10 km wide windows were collected. Numbers represent distance in km from the origin along the axis.

Although numerous types of foliated rocks are present in the terrane (e.g. amphibolites, schists, gneisses and granites), foliation parallel fracture spacing is wider in the massive gneissic rocks of the Nashoba Formation than in the multilayered Marlboro Formation. Amphibolites, schists and gneisses are interlayered in the Marlboro Formation, thus causing narrower fracture spacing than in massive rocks. In general, joint spacing decreases from the southwest to the northwest of the terrane. This is a reflection of change in rock type where widely spaced joints are common in the metamorphic and igneous rocks that are prevalent in the southwest of the terrane. In contrast, fracture spacing is narrower in the plutonic and volcanic rocks found in the northeast of the terrane.

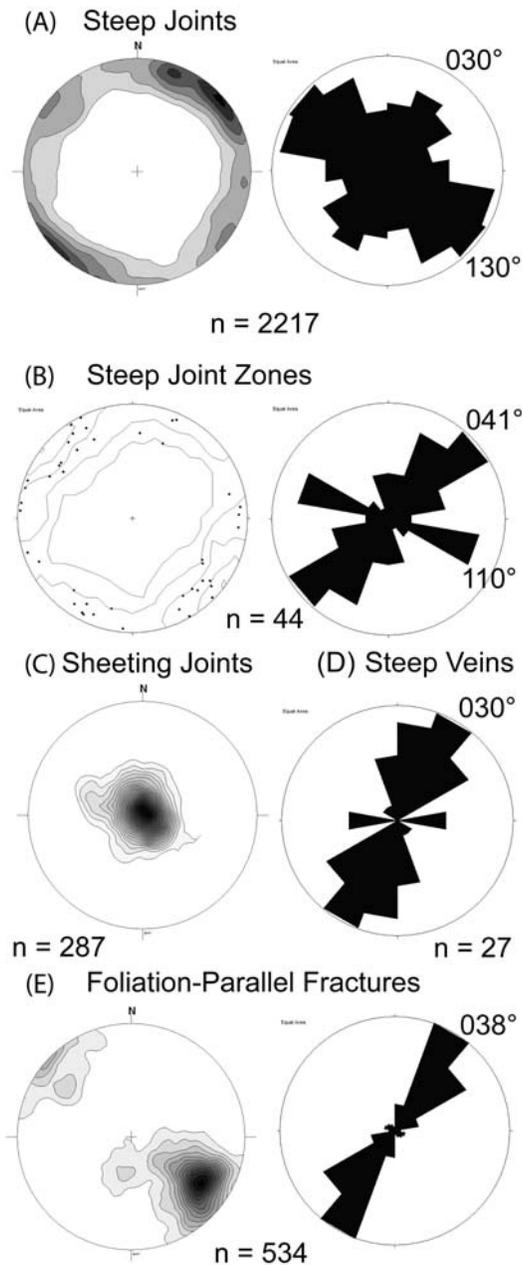


Figure 2. Contour stereoplots (lower hemisphere, equal area) and rose diagrams showing the orientations of different fracture types. (A) 1% area-contour plot of poles to joints with steep dips. Rose diagram shows two major sets trending $\sim 030^\circ$ and $\sim 130^\circ$ are prominent. (B) Kamb contour plot of poles to joint zones with steep dips. Contour interval = 2.0, and significance level = 3σ . Rose diagram shows two prominent sets. (C) 1% area-contour plot of poles to sheeting joints. (D) Rose diagram of steeply dipping veins trending $\sim 030^\circ$. (E) 1% area-contour plot of poles to FPFs and rose diagram showing fractures trending $\sim 038^\circ$.

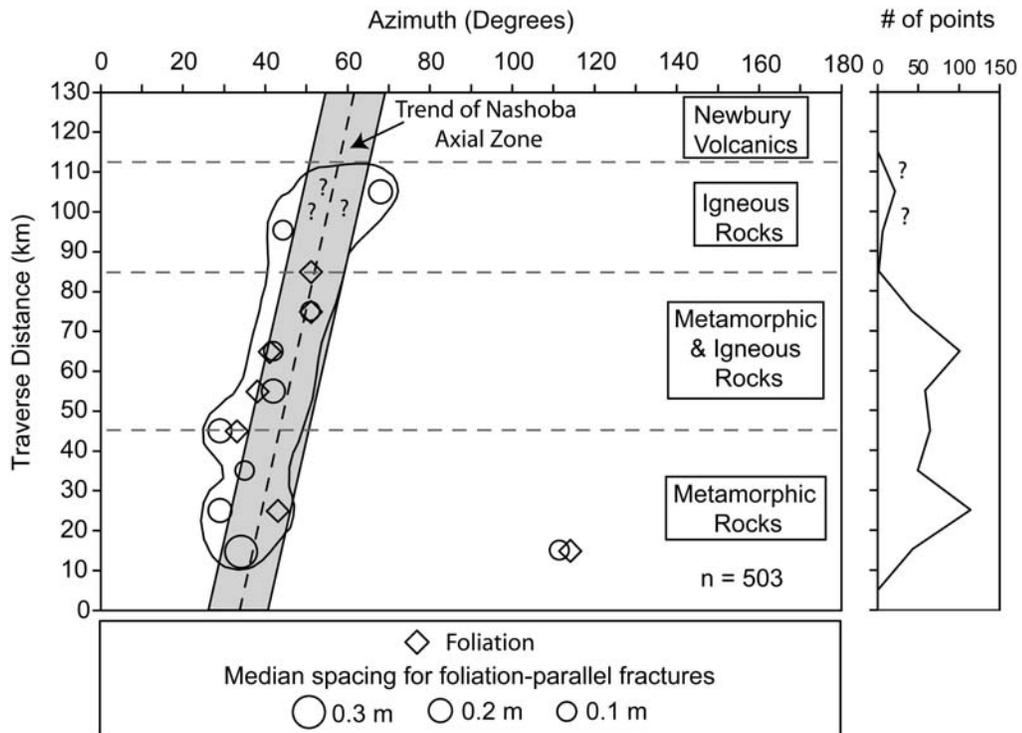


Figure 3. Azimuth versus traverse distance (AVTD) plot for FPFs with steep dips. The general trend of the FPFs and foliation sub-parallel the trend of the Nashoba axial zone. The spacing of FPFs is generally narrow.

Although fewer foliation parallel fractures than joints were collected in this study, the results show that foliation parallel fractures have comparable trace-lengths to steep joints. The median trace length of all foliation parallel fractures and steep joints is equal to 1.5 m. The median trace lengths of foliation parallel fractures also appear to vary as a function of the degree of development of foliation. Lithologic units with moderately/strongly developed foliation (e.g. Nashoba and Marlboro Formations) have the longest foliation parallel fractures. On the other hand, units with weakly developed foliation (e.g. Andover Granite and Sharpners Pond Diorite) have the shortest trace lengths of foliation parallel fractures.

Numerical simulations of groundwater flow in synthetic fracture network created with statistics derived from the Nashoba terrane were conducted to evaluate the role that foliation parallel fractures play in conducting fluids in the subsurface. The results reveal that foliation parallel fractures may contribute up to 30% of the flow in the networks. Furthermore, the potential for recharge is increased in rock units with foliation parallel fractures because they provide additional conduits along which fluids can percolate to the subsurface. Foliation parallel fractures are thus important to groundwater flow because they contribute significantly to the flow and storage properties of fractures in crystalline rocks.

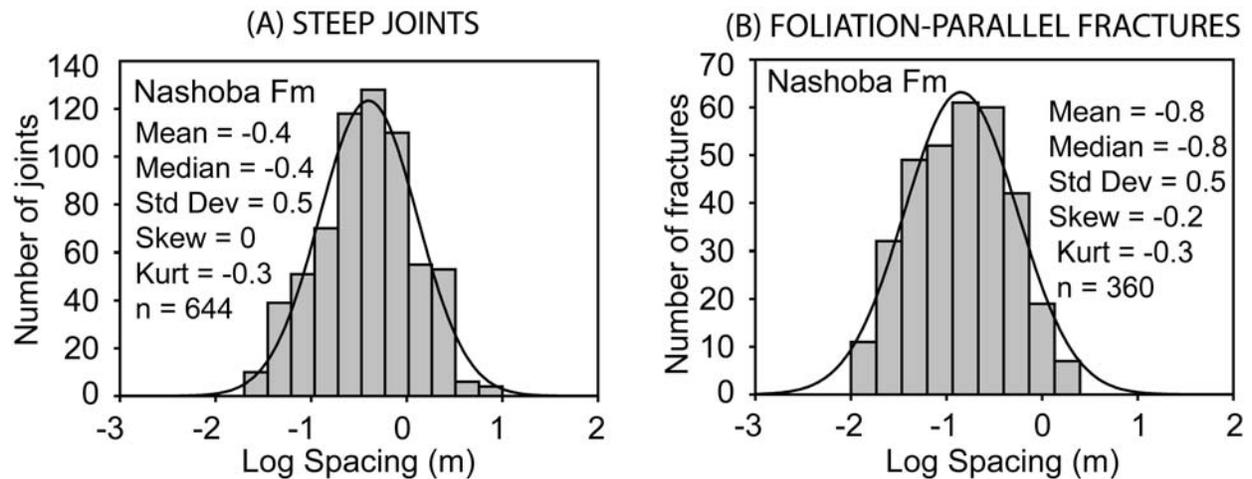


Figure 4. Examples of frequency distribution plots of the spacing of (A) joints, and (B) FPFs derived from the Nashoba Formation. Note that the statistics are in log base 10.

Contrasting various fracture sampling techniques from layered rocks

Three fracture sampling techniques were tested against each other to evaluate the effectiveness of each method in recreating natural fracture networks in lower Silurian dolomites of northeastern Wisconsin. An image of a quarry wall showing the fractured and layered dolomitic units was used as a template to map fractures using GIS software (Fig. 5a). A full description of the site and fracture characteristics can be derived from Underwood et al. (2003) who are the original workers at the site. The methods that were tested in this study are (1) the single scanline (SSM), (2) selection (SM) and, (3) multiple scanline methods (MSM). Two scenarios were tested for the single scanline method. The first uses a scanline with a pitch of 20° whereas the pitch for the line in the second scenario is 50° (Fig. 5b). The reason for using two lines was to assess the influence of the location of the scanline with respect to the fractures being sampled. In the selection method, three sites on the quarry wall were selected where fracture characteristics were collected (Fig. 5c).

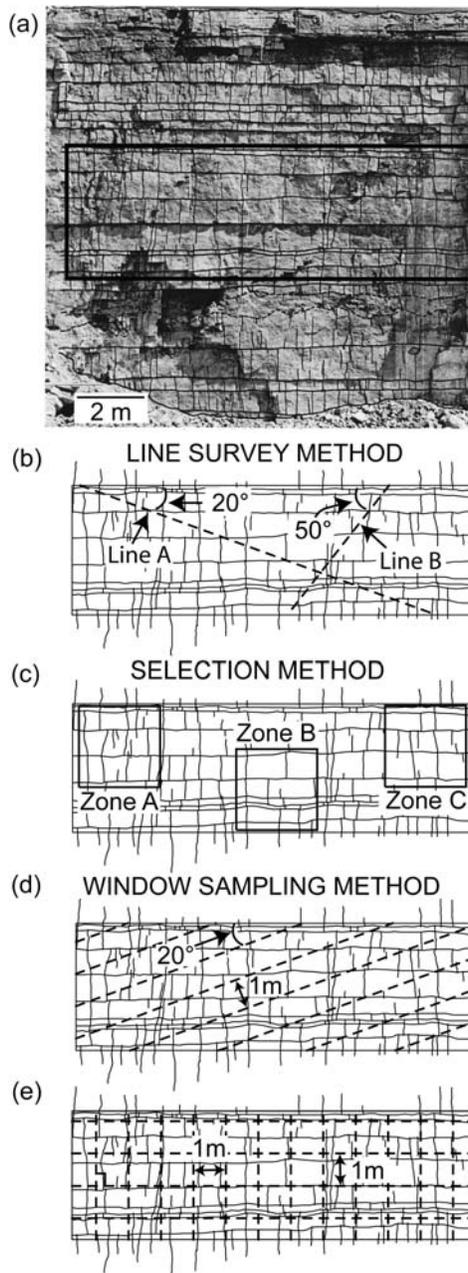


Figure 5. (a) Image of subvertical fractures abutting horizontal stratigraphic horizons was used as a template for digitizing fractures in a GIS. (b) Single scanline survey: Line traverses (dashed lines) used to sample fractures (solid lines) from sets. (c) Selection method: Fracture data that were collected from the three zones A, B and C were lumped together to create a fourth set. (d) Multiple scanline method: A set of multiple traverse lines (dashed lines) used to sample fractures within a window. (e) Multiple scanline method: Two sets of traverse lines (dashed lines) placed orthogonal to both fracture sets. The data from all three zones (A, B, C) were then lumped together to form a fourth data set. In so doing, an evaluation of the quality of the data collected at random sites on the outcrop could be made. The multiple scanline method consists of placing multiple scanlines on the quarry wall at various angles to the fracture sets being measured (Fig. 5d and e). A summary of the classification system is shown in Figure 6.

#1 SSM Fig. 5b	#2 SM Fig. 5c		#3 MSM Fig. 5d
BQ#1A Line with ~20° pitch	BQ#2A All fractures in Zone A	BQ#2C All fractures in Zone C	BQ#3A Multiple lines with ~20° pitch
BQ#1B Line with ~50° pitch	BQ#2B All fractures in Zone B	BQ#2D Zones A-C Lumped	BQ#3B Sets of multiple lines normal to fracture sets

Figure 6. Classification scheme employed for various versions of all sampling techniques utilized at the site (SSM = single scanline method, SM = selection method, MSM = multiple scanline method).

Fracture data collected using each sampling technique were used to stochastically create synthetic fractures in 10 x 10 x 10 m box regions (Fig. 7) using a procedure adapted from Caine and Tomusiak (2003). Another three-dimensional DFN model with specific locations, orientations, and characteristics of each fracture was generated for the real world fracture sets (i.e. the deterministic model). Groundwater was then simulated to flow in the fracture networks in three mutually perpendicular directions. Porosity and potential permeability estimates were computed for each of the stochastic and deterministic models to evaluate the effectiveness of each sampling technique in capturing the essential characteristics of each fracture network. Potential porosity error (i.e. percentage change between the deterministic and stochastic porosity estimates) was then computed (Fig. 8). The results show that the errors associated with the selection and multiple scanline methods were within acceptable ranges of arbitrary chosen thresholds ($\pm 10\%$ porosity error). The single scanline method had a large range of relative error ($\sim 3\%$ - 19%). This result indicates that the single scanline method is highly sensitive to the location of the scanline.

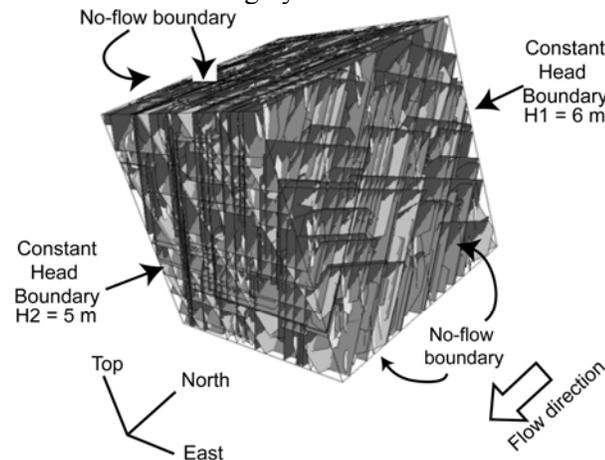


Figure 7. An example of the set up and boundary conditions used to simulate groundwater flow in discrete fracture networks. In this example, groundwater was simulated to flow in fractures from north to south under a hydraulic gradient of 0.1. Flow in other directions was simulated by switching the boundary conditions of the 10 x 10 x 10 m model region.

The selection and multiple scanline methods are more consistent in recreating the fracture network void space than the single scanline method.

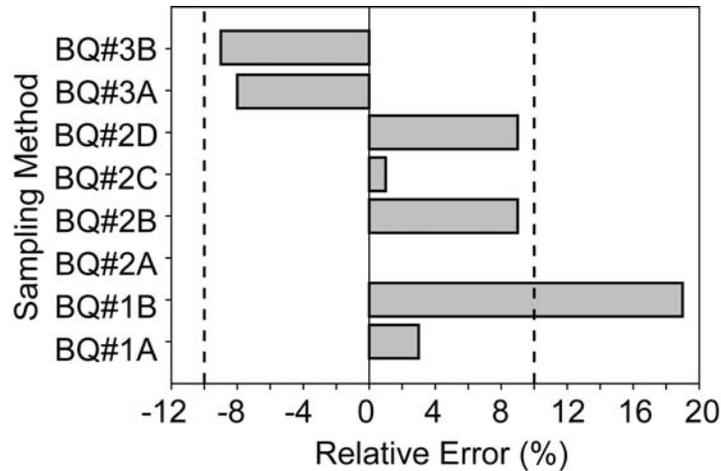


Figure 8. The relative error between porosity estimates derived from deterministic and the mean of 10 randomly selected stochastic DFN models. A negative value means that stochastic porosity is lower than deterministic porosity and vice versa. Dashed vertical lines represent range of acceptable relative error ($\pm 10\%$).

Potential permeability ratios, R (where R is the ratio of the stochastic permeability to the deterministic permeability) derived from models created with data collected by each technique show that most of the techniques produced models with R values between arbitrary thresholds of 0.8 and 1.2. This result suggests that permeability estimates may be within acceptable ranges even though the total void space in the model is not correct. Thus, porosity and potential permeability ought to be used together in assessing DFN models. Since the porosity for the single scanline method is highly variable, emphasis for determining which of the methods is more robust will be placed on the selection and multiple scanline methods. However, in practice placing multiple scanlines along an outcrop may be impractical or impossible. And where possible, the procedure may be quite time consuming. Thus, the selection method is recommended as the fracture sampling method of choice because it gives almost as good results as the multiple scanline method while requiring less effort and time to implement. A manuscript describing the study above (Manda and Mabee) has been submitted to Hydrogeology Journal and is currently being evaluated.

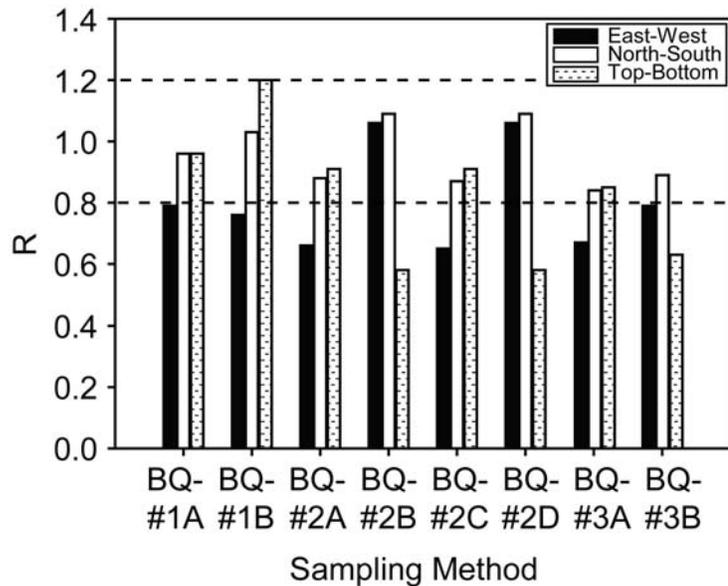


Figure 9. R results computed from stochastic models. Dashed horizontal lines represent upper and lower thresholds of 0.8 and 1.2, respectively ($R = K_s/K_d$). If $R > 1$, K_s overestimated; If $R < 1$, K_s underestimated.

Ongoing Work: The Hydrostructural Domain Revisited

The concept of the hydrostructural domain is fairly new. Mackie (2002) was the first to define hydrostructural domains describing them as packages of rocks where fracture intensity (the number of fractures per unit length) is different from adjacent rock units. Since this initial description, a few papers have been published that document the delineation of hydrostructural domains in fractured rock terranes (e.g. Surrrette et al., 2007, Surrrette and Allen, 2008). These workers have proceeded to numerically model and assign unique hydraulic properties to hydrostructural domains that have been compared to field derived pumping test data. Despite the apparent similarity between modeled and field derived properties, there are a few problems associated with delineating and assigning hydraulic properties to packages of rocks based solely on fracture intensity.

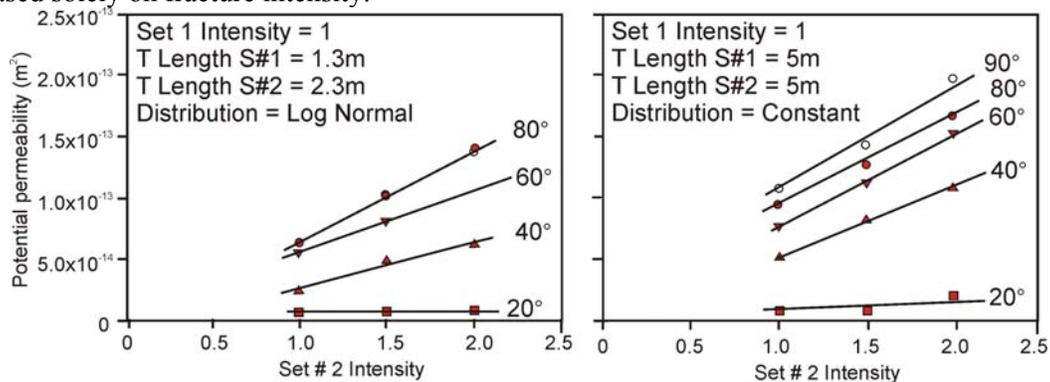


Figure 10. Plots of the intensity of fracture set #2 versus potential permeability derived from numerical simulations. The angles of intersection between sets 1 and 2 are given in degrees for each graph. Although the plots were created with different fracture attributes and distributions, the general trends in are similar. Note that the potential permeability is more sensitive to the angle of intersection than to the intensity of the second set.

First, hydrostructural domains do not take into account the influence of fracture trace length on permeability. Longer fractures are more likely to encounter other fractures, particularly where fracture orientations are not the same, thereby increasing fracture network connectivity. Second, the number of fracture sets and the relative orientations of these sets affects the connectivity of the DFN. This is because fracture network permeability is controlled in part, by fracture connectivity. For example, where two fracture sets are present, fracture connectivity increases to a maximum when the trends of the two sets are mutually perpendicular (Fig. 10). All things being equal, the bulk permeability of a DFN is at a maximum when the connectivity is also at a maximum. Therefore, two areas that have the same intensity but different angles of intersection between fracture sets will have significantly different permeability estimates.

The major problem with the current application of hydrostructural domains is that (1) there is lack of inclusive criteria that can be used to define hydrostructural domains and, (2) pre-established categories of hydrostructural domains are not available. This has resulted in arduous investigations at each site just to define a domain. The domains described at such sites are typically unique, and therefore not easily transferable to other sites with potentially similar fracture distributions.

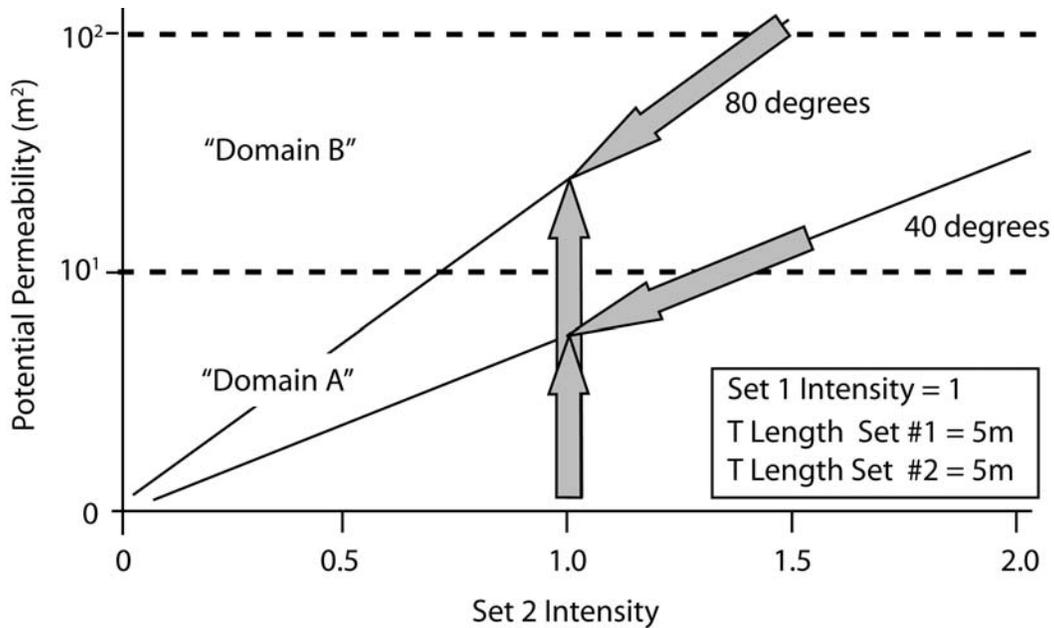


Figure 11. Example of the procedure suggested to determine the domain representing a fracture network with two fracture sets intersecting each other at either 80° or 40°. If the second set has an intensity of 1, then the domain will depend on the angle of intersection between the fracture sets.

The ongoing research incorporates fracture trace length, number of fracture sets and relative fracture orientations to re-define hydrostructural domains. Numerical modeling techniques are used to build DFNs that may represent field derived fracture distributions. The FracMan suite of software packages is used to run Monte Carlo type simulations to derive permeability estimates of fracture networks. Sets of given fracture parameters that produce DFNs with particular ranges of permeability will be used to classify hydrostructural domains. Since it has already been established that aperture plays a big role in determining the transmissive properties of fractures, it is assumed in this study that the aperture distribution for all fracture sets is constant. A constant aperture is commonly assumed when evaluating the role of other fracture characteristics in channeling fluids in fracture networks (e.g. de Dreuzy et al., 2001; Min et al., 2004) because aperture is so difficult to measure in the field.

Figure 11 shows a possible procedure that might be employed to determine which domain a given fracture network belongs to. Assuming that there are two fracture sets (intensity of fracture set #1 = set#2 = 1), and each fracture set has a median trace length of 5 m, the first thing to do would be to determine the angle of intersection between the two fracture sets and then follow the line on the plot to where it intersects the line x (set 2 intensity) = 1. If the angle of intersection is 40°, then the network would be in domain A. If however, the angle of intersection is 80°, the fracture network would be in domain B, as determined by the point of intersection between the 80° line and line $x = 1$. In this example the domain cutoff is an order of magnitude.

As might be expected, there are an infinite number of outcomes for plots such as those shown in Figure 11. Fracture characteristics such as trace length and orientation will be chosen in given multiples so that the number of plots is reduced. These plots will thus represent upper bounds of the hydraulic property being sought. Multiple scenarios are currently being generated to create plots that can be used to define the domain fields. The advantage of this new approach is that the procedure is transferable and it allows a fairly rapid assessment of sites with fracture networks. A classification of the observed fracture network into a predetermined hydrostructural domain can then be used to infer the subsurface distribution of fractures, which can, in turn, be related to how well the rock unit transmits and stores water.

USING HYDROMORPHOLOGICAL SIGNATURES TO DETERMINE FLOW RELATED HABITAT THRESHOLDS FOR INSTREAM COMMUNITIES

Basic Information

Title:	USING HYDROMORPHOLOGICAL SIGNATURES TO DETERMINE FLOW RELATED HABITAT THRESHOLDS FOR INSTREAM COMMUNITIES
Project Number:	2006MA60B
Start Date:	3/1/2006
End Date:	2/29/2008
Funding Source:	104B
Congressional District:	1st
Research Category:	Biological Sciences
Focus Category:	Hydrology, Ecology, Management and Planning
Descriptors:	
Principal Investigators:	Scott D Jackson, Christina Cianfrani, Piotr Parasiewicz

Publication

1. Legros, Jeffrey, Piotr Parasiewicz, 2008. Recent innovations and applications of the target fish community (tfc) approach: 2000 – 2008. Poster at 5th Massachusetts Water Resources Research Conference, UMass Amherst, April 8, 2008.
2. Cianfrani, C.M., P. Parasiewicz, J. Legros, and M. Wirth, 2007, Using Hydromorphological Signatures to Determine Flow Related Habitat Thresholds for Instream Communities, Poster at Sustainable Waters in a Changing World: Research to Practice, 4th Annual Conference of the Massachusetts Water Resources Research Center, Amherst, MA. April 2007.

**Using Hydromorphological Signatures to Determine
Flow Related Habitat Thresholds for Instream Communities**

**Massachusetts Water Resources Research Center Grant
Final Report – June 2008**

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INTRODUCTION

Field measurements from the existing database of streams in the northeastern United States were used to evaluate the feasibility of using hydromorphological (HMU) signatures in determining fish communities as part of an overall methodology for quantifying instream flow requirements and habitat thresholds. The results of this research may lay the foundation for using HMU signatures to identify thresholds of change in aquatic communities as a result of changes in hydrologic regime due to water withdrawals/alterations. These thresholds could then begin to provide the scientific basis for determining acceptable limits of hydrologic change within river systems to protect ecological integrity.

Our project builds upon a newly developed French method (Le Coarer, 2005) of using hydraulic (velocity and depth) distribution score-cards, called “Hydrosignatures,” as a habitat metric. We apply this concept to represent the distribution of HMUs in the stream for different flow conditions (e.g. high, medium, low). We then attempt to use these HMUs to create templates that can be used with fish habitat models in an attempt to predict the probable composition of fish communities associated with these patterns. This final report presents the results from both the first and second years (Phase I and II) of the study (Phase I).

PHASE I (Year 1)

The purpose of Phase I of this project was to use existing data to show proof of concept of a method to: 1) identify and map HMU signatures for river sections under different flow conditions; and 2) relate the HMU signatures to physical habitat. To accomplish this, data including habitat and HMU mapping, flow-duration curves, and fish habitat models (generated using MesoHABSIM) were used to compute the relative area available for habitat for individual species under varying flow conditions (high, medium, and low summer flows) (Figure 1). This was completed for both existing summer flow durations as well as modeled pristine flow conditions.

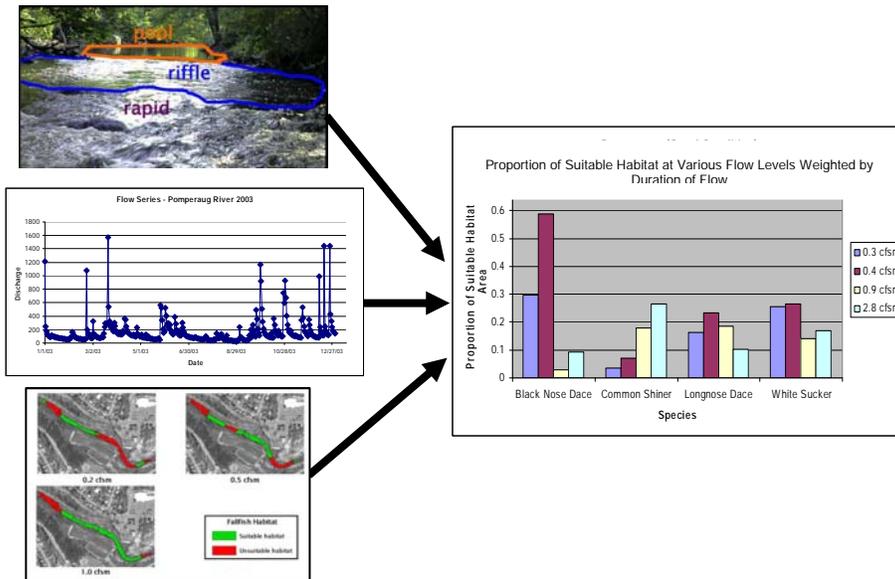


Figure 1. Basic methodology to create habitat probability models under various flow conditions.

Hydraulic and Fish Data

As part of previous projects, HMUs were mapped in the field for 10 rivers in Connecticut, Massachusetts, New Hampshire, and New York. Each HMU was mapped using a personal digital assistant (PDA) and ArcPad software (ESRI, Redlands, CA). Aerial photographs uploaded to the PDA were used to help identify river locations. Eleven HMU categories were used when mapping with definitions taken from Parasiewicz (2001): 1) backwater; 2) pool; 3) plungepool; 4) glide; 5) run; 6) fastrun; 7) rapids; 8) sidearm; 9) cascade; 10) riffle; and 11) riffle. Within each HMU, random velocity and depth measurements were taken.

Fish were collected using a backpack electro-shocker and a grid technique described by Bain et al. (1985). Sampling occurred in representative HMUs at each site on each river to ensure each type of habitat was appropriately represented. Fish were measured and identified to species.

Considerable effort was spent in year 1 of the project mining data from existing projects. Specific river sections were chosen according to project criteria. Data was then formatted for compatibility.

Habitat Suitability

Sites on the Quinebaug and Pomperaug Rivers were used to test the ability of the technique to detect differences in suitable habitat availability based on changes in flow regimes. Using four key species (as defined by the target fish community identified for the Quinebaug River), changes in habitat availability were modeled for four summer flow levels under two flow regimes, measured and 'pristine' (Figure 2). The regimes differed in percent duration of low, medium, and high flows.

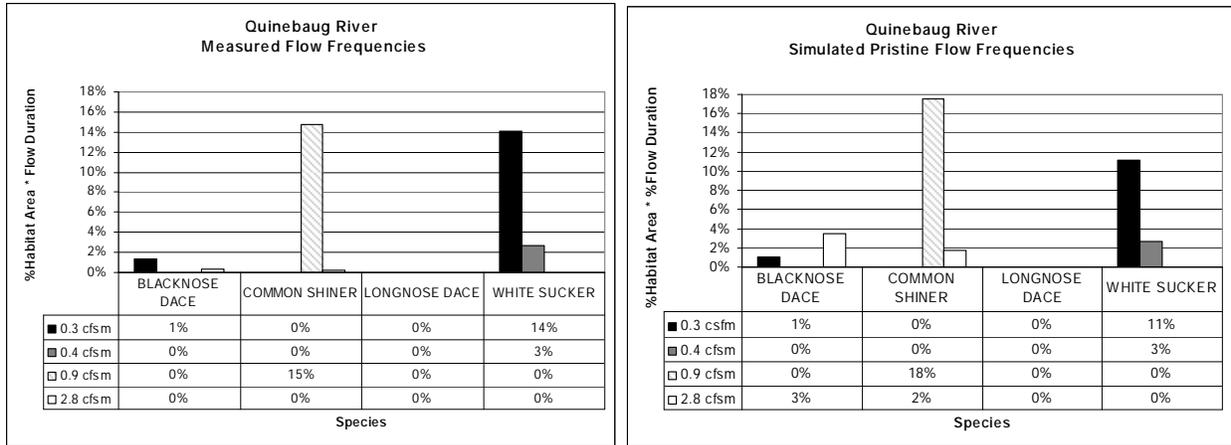


Figure 2. Available habitat (as percentage of total wetted width) for the Quinebaug River under two flow regimes weighted for duration during the summer.

The amount of available habitat was not sensitive to significant changes in flow regimes using this technique. Further analysis is needed to determine which component(s) may need adjustment in order to detect the differences. For example, research as part of another project has shown the choice of fish habitat model can have a significant impact on overall results. This study compared the predictive capability of models developed using: 1) three rivers individually (each with differing levels of impairment); 2) a regional model using significant parameters from all three rivers; and 3) a global model using all field collected data for all rivers. Such considerations will be explored as the model is refined.

HMU Classification

We explored the possibility of reducing the number of HMUs through cluster analysis. We analyzed trends among the high, medium, and low flow data of the HMUs used in the field mapping protocol. We aim to develop a standardized characterization, or template, of depth and velocity for each HMU to use in fish habitat models. If templates can be developed based on HMUs, field work effort could be reduced significantly (i.e. one would only have to map the HMU and take a minimal number of depth and velocity measurements). More than one potential “template” may result if distributions vary for different flow levels.

Preliminary k-means hierarchical cluster analysis (McGarigal, et al, 2000) was used to reduce the number of HMUs. The analysis using depth and velocity measurements showed a reduction was possible in the number of HMUs from 11 to 8. This analysis resulted in the following HMUs: 1) backwater; 2) pool; 3) glide/run; 4) plungepool; 5) sidearm; 6) cascade; 7) ruffle/riffle; and 8) fastrun/rapids

For the second part of this analysis, histograms for the depth and velocity measurements for each HMU for each flow (high, medium, low) were created. Bins were predetermined as per NEIHP protocol with bin size for depth equal to 25 cm and for velocity equal to 15 cm/s. The histograms were standardized and plotted to inspect for visual trends (Figure 3). Visual inspection was followed with Kolmogorov-Smirnov tests (Davis, 2002) in a pairwise fashion for all combinations of the three flow data sets within each HMU (i.e. low vs medium, medium vs high

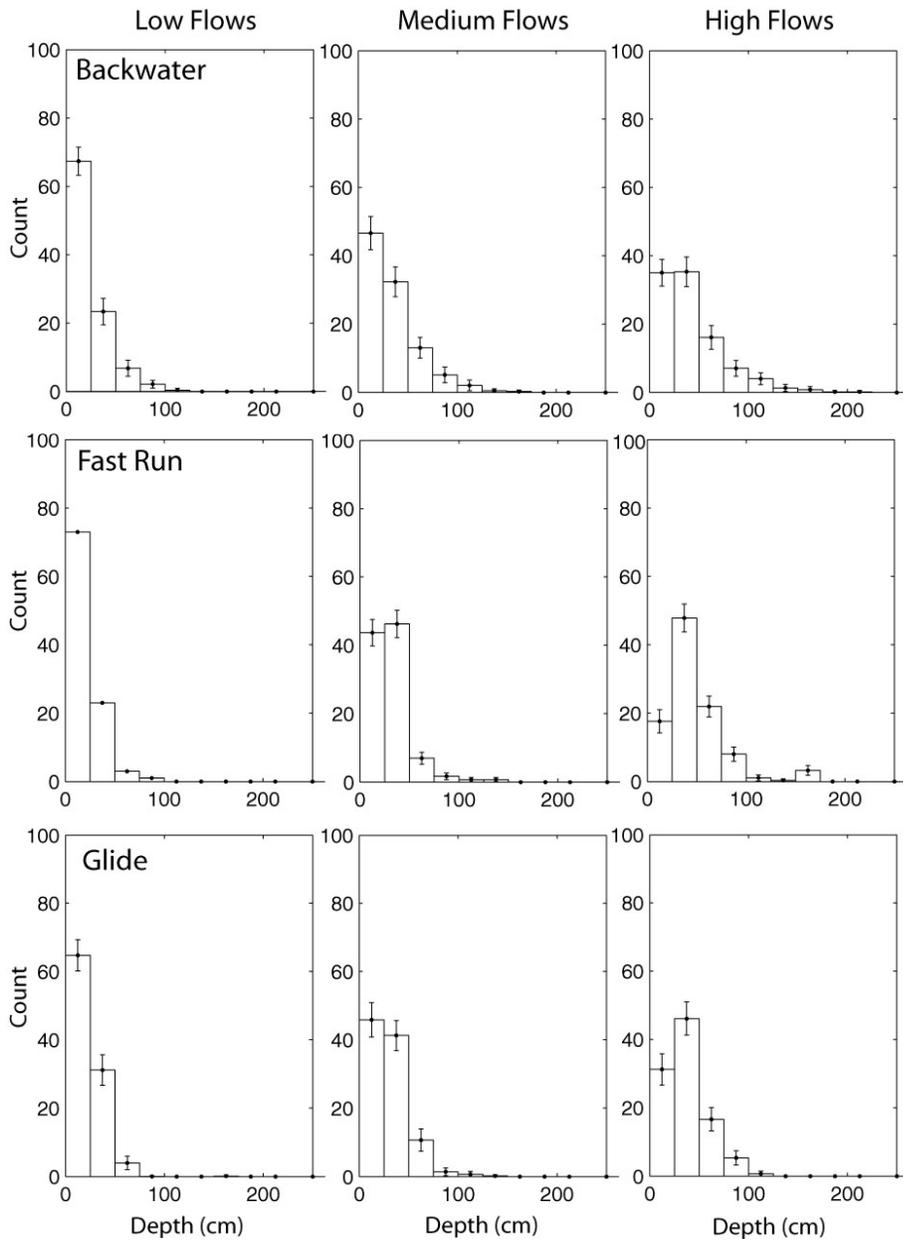
and high vs low). This test was used to determine which data sets could be combined. This was repeated for both depth and velocity data. Preliminary results show that few data sets can be combined and that templates for each HMU for each flow will most likely be necessary.

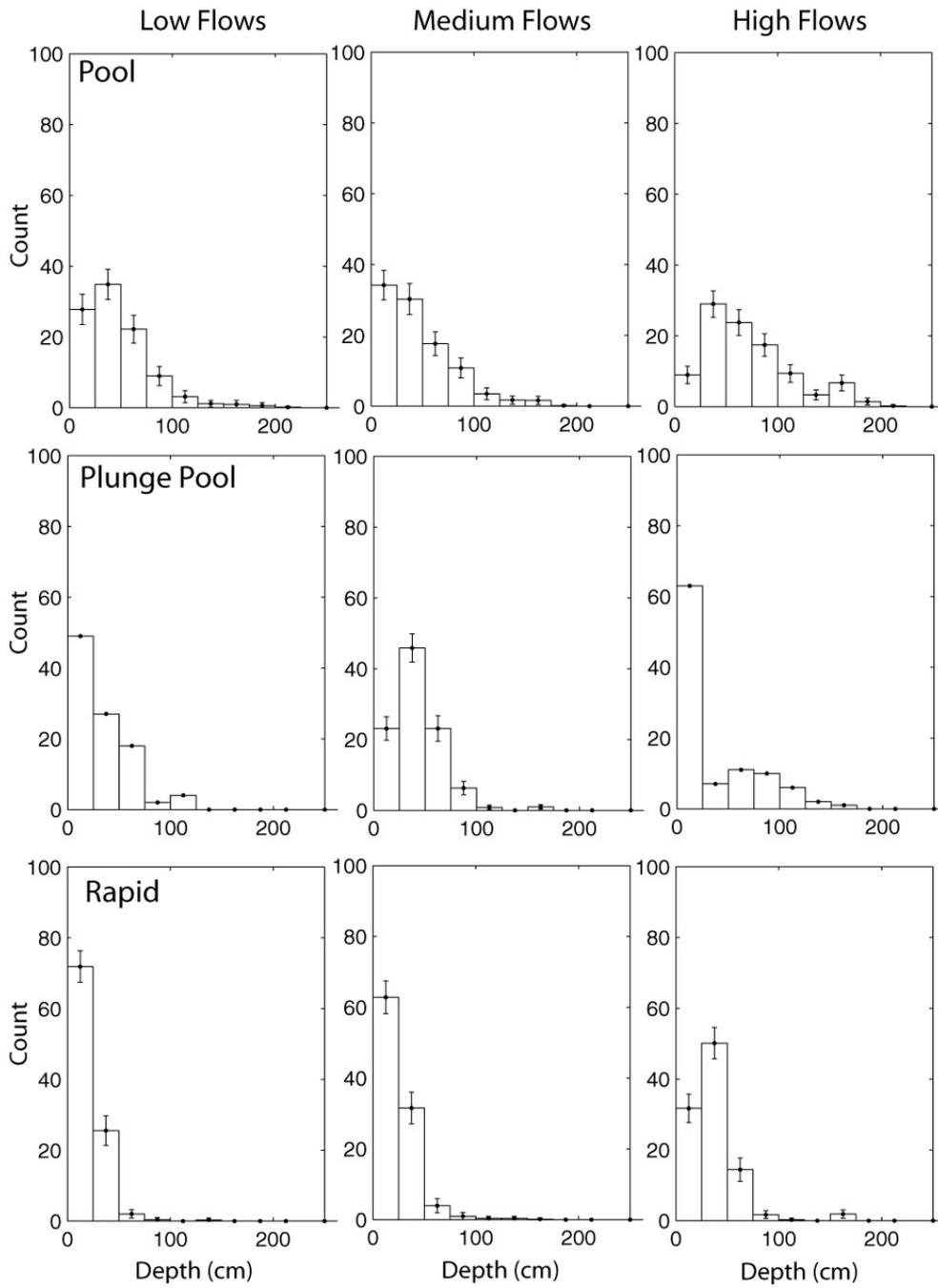
PHASE II (Year 2)

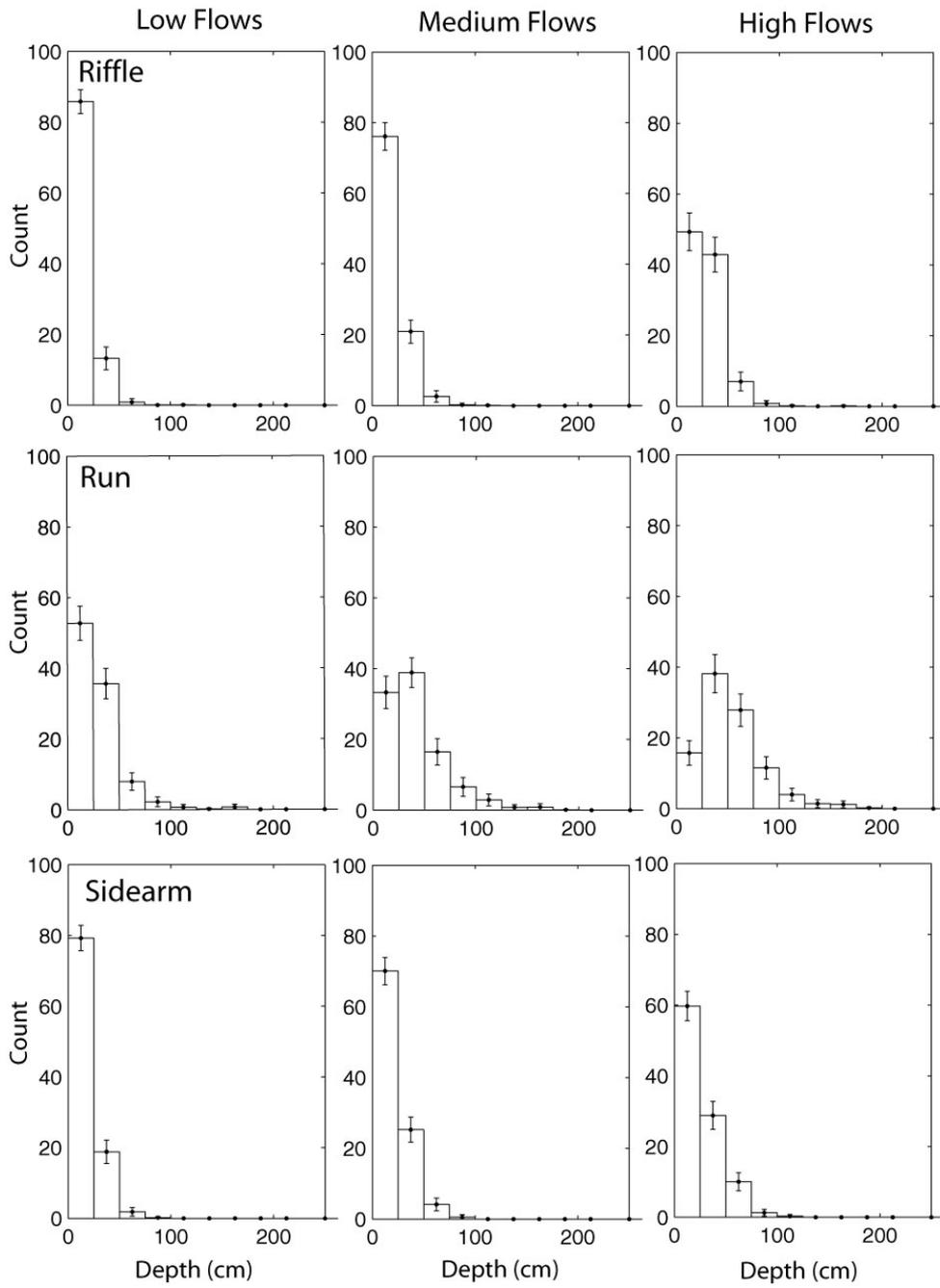
In the second year of the project, we continued evaluating the modeling approach outlined in Phase I. We completed extensive statistical analyses that: 1) clustered and reduced the number of HMU categories; 2) identified distribution curves for the reduced number of HMUs across 3 different flows; 3) quantified the similarities and differences in distributions across flows for a given HMU; 4) found distribution fits for a given HMU within each flow; and 5) generated signature plots for paired data and quantified the statistics differences. The results of these analyses are found below.

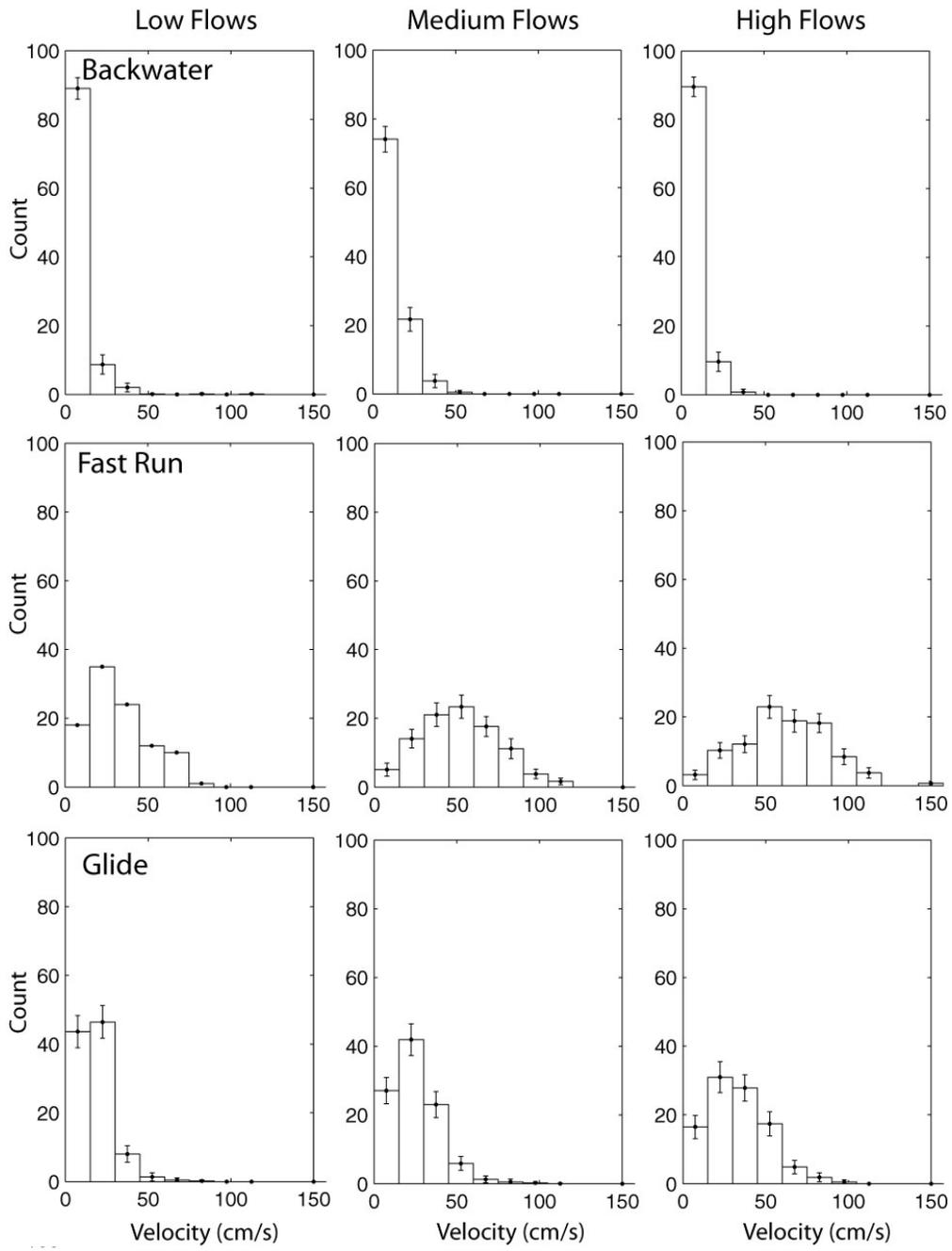
Steps 1) and 2): Generate distribution curves, aggregating cascade and rapids (Rapids) and ruffle and riffles (Riffles)

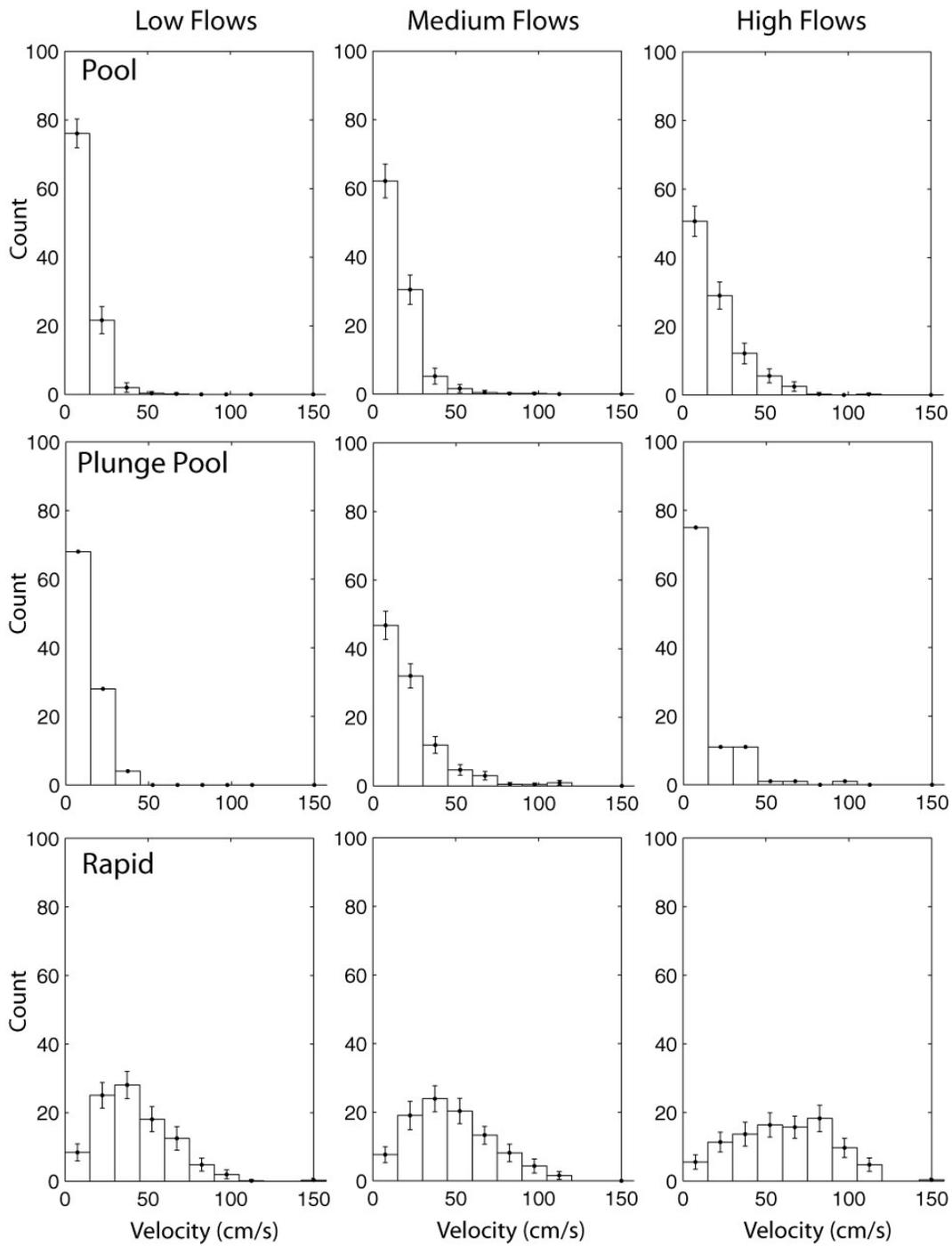
The following 6 figures (3 for depth, 3 for velocity) represent depth and velocity distributions for the three specified flow condition (low, medium, high flows) across 9 defined HMUs. A pre-specified bin spacing was used for either depth (every 25 cm) or velocity (every 15 cm/s). This analysis was conducted by grabbing 100 random samples for each flow within each HMU. This was repeated 100 times (100 bootstraps), and an average was computed within each bin along with 2 x standard deviation around this average, denoted on the figures with the bar height and error bars, respectively.











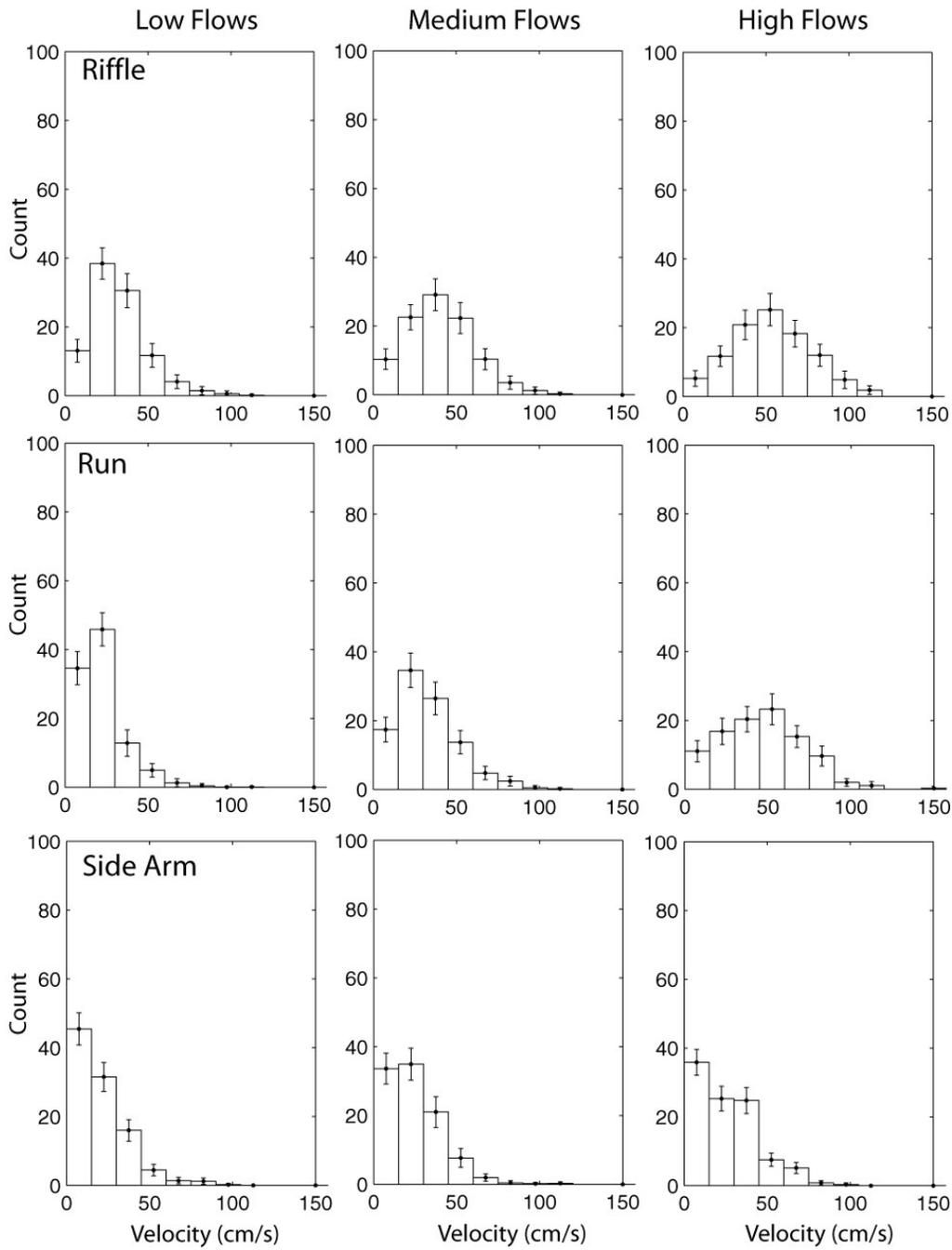


Figure 3. Velocity and depth distributions for each of 9 HMUs for each of 3 flows.

Step 3): Quantify similarities and differences in distributions across flows for a given HMU.

Differences amongst flows were quantified for each HMU using the Kruskal-Wallis nonparametric test, followed by pairwise Mann-Kendall analysis when significant differences were found. With the exception of Side Arm depths, there were significant differences between at least one flow grouping depth and velocity distributions. Pairwise analysis of depths indicated no significant differences between low and medium flows for glides, pools, and riffles nor no significant differences between medium and high flows for backwater and runs (Table 1). For velocity distributions, pairwise analysis indicated no significant differences between low and medium flows for pools and rapids, along with no significant differences between medium and high flows for pools and Side Arm HMUs (Table 2)

Table 1. Comparison of depth distributions between flows for a given HMU.

HMU	n	Mean Score			X ² Statistic	Prob>X ²
		Low Flows	Med Flows	High Flows		
Backwater	100	117.0 ^a	162.9 ^b	171.6 ^b	22.8	<i>p</i> <0.0001
Fast Run	100	93.6 ^a	146.0 ^b	211.8 ^c	93.1	<i>p</i> <0.0001
Glide	100	123.4 ^a	135.0 ^a	193.1 ^b	37.0	<i>p</i> <0.0001
Pool	100	132.2 ^a	137.8 ^a	181.5 ^b	19.4	<i>p</i> <0.0001
Plunge Pool	100	144.1 ^a	181.2 ^b	126.2 ^a	21.4	<i>p</i> <0.0001
Rapid	100	111.0 ^a	147.9 ^b	192.7 ^c	44.6	<i>p</i> <0.0001
Riffle	100	113.3 ^a	143.1 ^a	195.1 ^b	45.6	<i>p</i> <0.0001
Run	100	97.8 ^a	162.0 ^b	191.7 ^b	61.3	<i>p</i> <0.0001
Side Arm	100	135.9	150.3	165.4	5.8	<i>p</i> <0.0551
^a Classifications with the same letter are not significantly different between flows ($\alpha=0.05$ level)						

Table 2. Comparison of velocity distributions between flows for a given HMU.

HMU	n	Mean Score			X ² Statistic	Prob>X ²
		Low Flows	Med Flows	High Flows		
Backwater	100	138.8 ^a	174.9 ^b	137.8 ^a	21.2	<i>p</i> <0.0001
Fast Run	100	94.4 ^a	163.1 ^b	194.0 ^c	69.0	<i>p</i> <0.0001
Glide	100	102.1 ^a	151.9 ^b	197.5 ^c	60.9	<i>p</i> <0.0001
Pool	100	125.1 ^a	150.2 ^{a,b}	176.2 ^b	18.0	<i>p</i> <0.0001
Plunge Pool	100	141.4 ^a	191.6 ^b	118.6 ^a	40.7	<i>p</i> <0.0001
Rapid	100	130.0 ^a	135.9 ^a	185.6 ^b	24.8	<i>p</i> <0.0001
Riffle	100	106.7 ^a	155.8 ^b	189.0 ^c	45.6	<i>p</i> <0.0001
Run	100	100.3 ^a	152.1 ^b	199.1 ^c	65.0	<i>p</i> <0.0001
Side Arm	100	124.4 ^a	160.3 ^b	166.8 ^b	14.1	<i>p</i> <0.0001
^a Classifications with the same letter are not significantly different between flows ($\alpha=0.05$ level)						

Step 4): Find a distribution fit for a given HMU within each flow.

These shape trends were further quantified by looking at the distributions under each flow condition. One random sampling of data (n=100) was exported to JMP and data were fit to normal and lognormal distributions. The goodness of fit statistics are summarized in Table 3-5. Because of the large number of data in each set, the distributions with $W > 0.95$ are considered normally distributed and $D < 0.1$ as lognormally distributed. Some distributions meet both these criteria, therefore, the ‘best fit’ between the two distributions is chosen. Depth measurement approximated lognormal distributions for Fast Run, Plunge Pool, Rapid, and Riffle for at least one of the three different flows. Other depth HMUs did not approximate normal or lognormal distributions (Tables 1-3). Velocity distributions, on the other hand, typically approximated one of the two distribution. For example, Backwater, Pool, Plunge Pools, and Side Arms fit lognormal distributions for most or all flows, while Riffles fit a normal distribution for all flows (Tables 3-6). Interestingly, velocity distributions for three HMUs, including Fast Run, Glide, and Runs, shifted from a lognormal to normal distributions from low to high flows (Tables 3-6).

Table 3. Normal and Lognormal distribution fits for low flows for a given HMU.

HMU	Normal				Lognormal			
	Depth		Velocity		Depth		Velocity	
	W	P<W	W	P<W	D	P>D	D	P>D
Backwater	0.80	$p < 0.0001$	0.46	$p < 0.0001$	0.07	$p > 0.1500$	0.84	$p < 0.0100$
Fast Run	0.90	$p < 0.0001$	0.95	$p = 0.0078$	0.12	$p < 0.0100$	0.14	$p < 0.0100$
Glide	0.93	$p < 0.0001$	0.91	$p < 0.0001$	0.10	$p = 0.0312$	0.29	$p < 0.0100$
Pool	0.63	$p < 0.0001$	0.87	$p < 0.0001$	0.05	$p > 0.1500$	0.38	$p < 0.0100$
Plunge Pool	0.87	$p < 0.0001$	0.84	$p < 0.0001$	0.33	$p < 0.0100$	0.43	$p < 0.0100$
Rapid	0.86	$p < 0.0001$	0.98	$p = 0.4763$	0.06	$p > 0.1500$	0.10	$p = 0.0307$
Riffle	0.71	$p < 0.0001$	0.97	$p < 0.0716$	0.11	$p < 0.0100$	0.10	$p < 0.0100$
Run	0.67	$p < 0.0001$	0.90	$p < 0.0001$	0.08	$p = 0.1456$	0.19	$p < 0.0100$
Side Arm	0.88	$p < 0.0001$	0.84	$p < 0.0001$	0.09	$p = 0.0405$	0.30	$p < 0.0100$

Table 4. Normal and Lognormal distribution fits for medium flows for a given HMU.

HMU	Normal				Lognormal			
	Depth		Velocity		Depth		Velocity	
	W	P<W	W	P<W	D	P>D	D	P>D
Backwater	0.87	$p < 0.0001$	0.73	$p < 0.0001$	0.09	$p = 0.0466$	0.60	$p < 0.0100$
Fast Run	0.93	$p < 0.0001$	0.90	$p < 0.0001$	0.07	$p > 0.1500$	0.08	$p = 0.1046$
Glide	0.93	$p < 0.0001$	0.94	$p = 0.0007$	0.10	$p = 0.0257$	0.19	$p < 0.0100$
Pool	0.91	$p < 0.0001$	0.87	$p < 0.0001$	0.10	$p = 0.0107$	0.37	$p < 0.0100$
Plunge Pool	0.86	$p < 0.0001$	0.83	$p < 0.0001$	0.08	$p = 0.0849$	0.21	$p < 0.0100$
Rapid	0.86	$p < 0.0001$	0.96	$p = 0.0032$	0.08	$p = 0.0986$	0.11	$p < 0.0100$
Riffle	0.79	$p < 0.0001$	0.96	$p = 0.0411$	0.08	$p = 0.1324$	0.12	$p < 0.0100$
Run	0.85	$p < 0.0001$	0.95	$p = 0.0015$	0.07	$p > 0.1500$	0.09	$p = 0.0734$
Side Arm	0.87	$p < 0.0001$	0.90	$p < 0.0001$	0.07	$p > 0.1500$	0.21	$p < 0.0100$

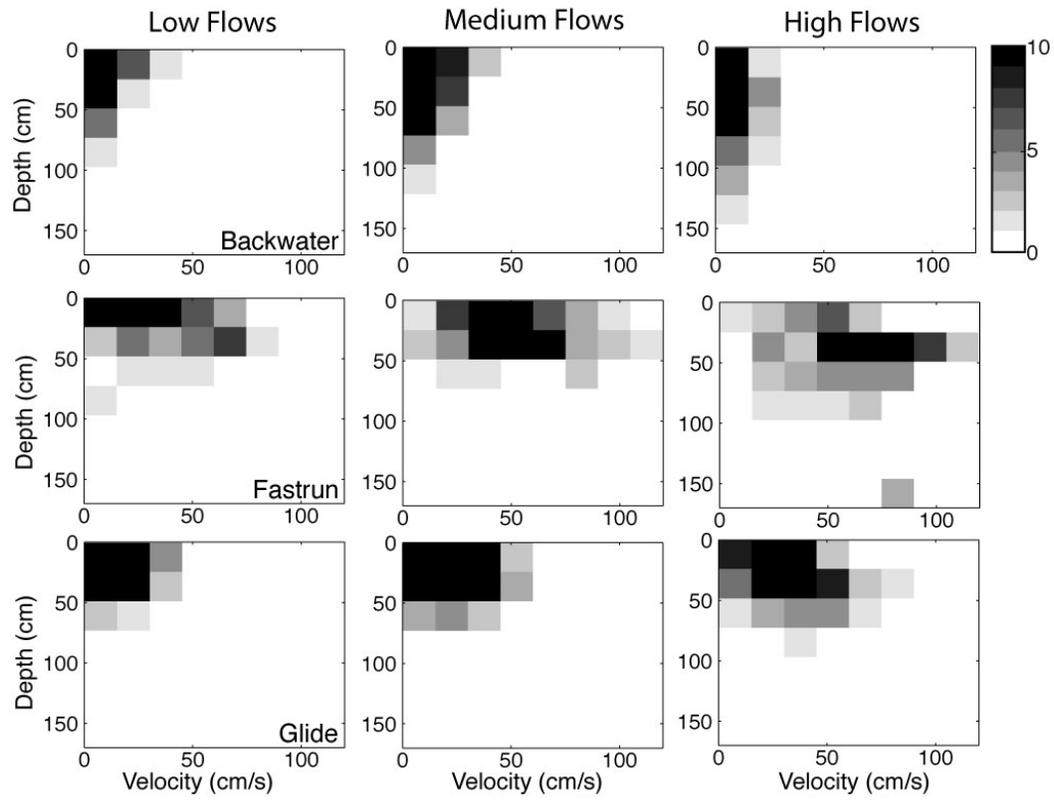
Table 5. Normal and Lognormal distribution fits for high flows for a given HMU.

HMU	Normal				Lognormal			
	Depth		Velocity		Depth		Velocity	
	W	P<W	W	P<W	D	P>D	D	P>D
Backwater	0.84	$p<0.0001$	0.47	$p<0.0001$	0.10	$p=0.0211$	0.84	$p<0.0100$
Fast Run	0.79	$p<0.0001$	0.98	$p=0.3170$	0.09	$p=0.0514$	0.10	$p<0.0100$
Glide	0.94	$p<0.0001$	0.97	$p=0.0886$	0.11	$p<0.0100$	0.09	$p=0.0494$
Pool	0.92	$p<0.0001$	0.88	$p<0.0001$	0.06	$p>0.1500$	0.27	$p<0.0100$
Plunge Pool	0.71	$p<0.0001$	0.60	$p<0.0001$	0.60	$p<0.0100$	0.73	$p<0.0100$
Rapid	0.70	$p<0.0001$	0.93	$p<0.0001$	0.11	$p<0.0100$	0.12	$p<0.0100$
Riffle	0.94	$p<0.0001$	0.98	$p=0.3815$	0.07	$p>0.1500$	0.08	$p=0.1318$
Run	0.89	$p<0.0001$	0.97	$p=0.0814$	0.07	$p>0.1500$	0.11	$p<0.0100$
Side Arm	0.87	$p<0.0001$	0.93	$p<0.0001$	0.08	$p=0.1070$	0.26	$p<0.0100$

Step 5): Generate signature plots (paired data) with the 9 HMU categories. Quantify statistical differences.

The signature plots were generated for the 9 HMU categories. The pre-specified bin spacing for either depth (every 25 cm) or velocity (every 15 cm/s) was used. 100 random samples for each flow within each HMU were used. This was done 100 times and summed the number of times a sample fell within each paired depth-velocity bin. The total number within a bin was divided by 100 to give a total % (therefore the graybar scale represents % of total within a bin).

In order to quantify differences in signature plots, a non-parametric statistical test was used to compare distributions between low, medium, and high flows for each HMU. This test, called the Wilcoxon Signed Ranks Test takes the paired observations, computes an absolute difference between observations (depth and velocity), and compares the ranks between these distributions. Results show that with the exception of Side Arms, there are statistical differences ($\alpha=0.05$) between flows for all other HMUs (Table 6). Some of these distributions shift with increasing flows (e.g. Backwater), while others are statistically different at either low (Fast Run, Riffle, Runs) or high (Pools) flows. The statistics confirm visual observations of the signature plots.



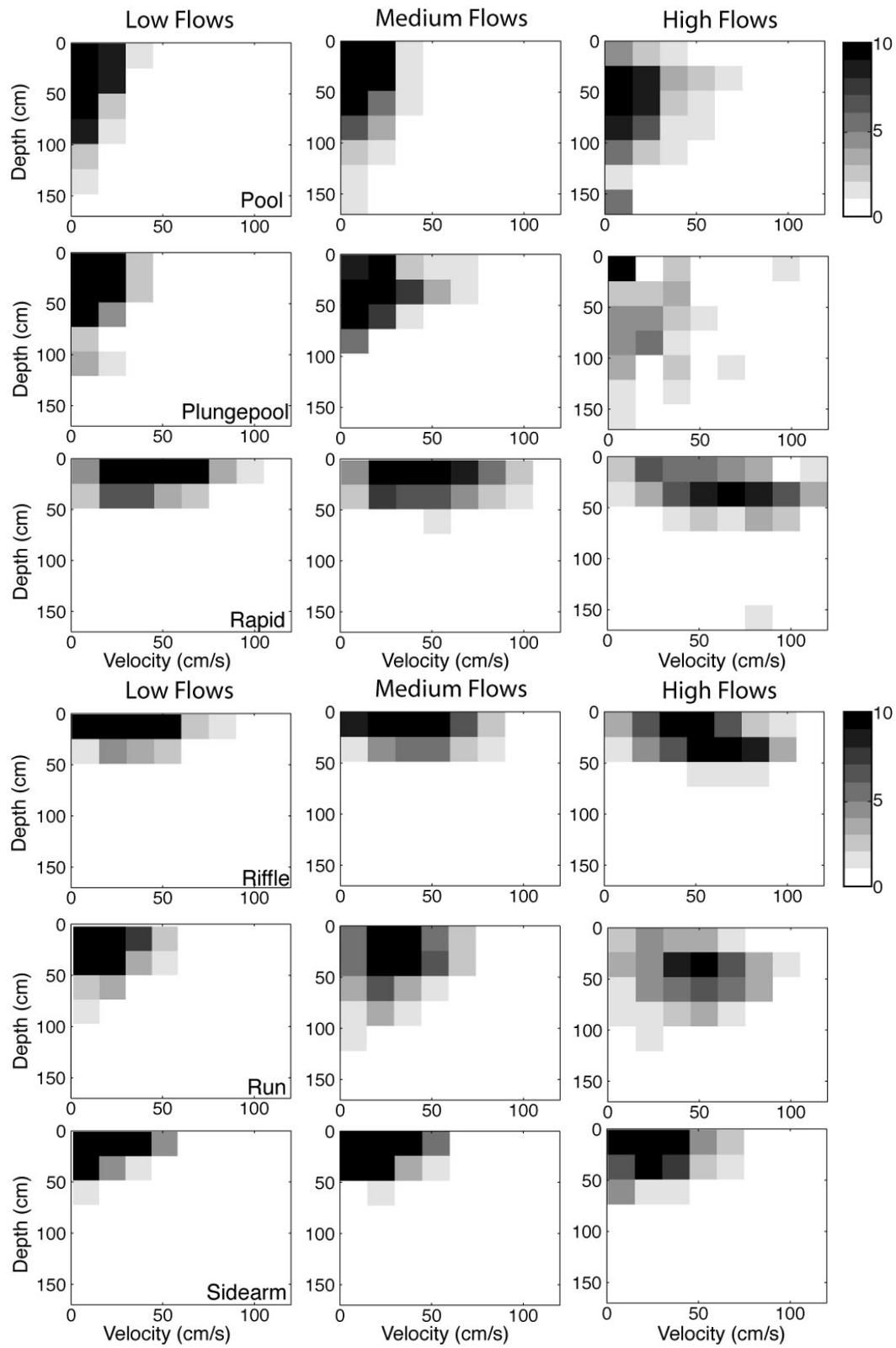


Table 6. Comparison of paired depth and velocity distributions between flows for a given HMU.

HMU	n	Mean Score		
		Low Flows	Med Flows	High Flows
Backwater	100	125.2 ^a	150.9 ^b	175.4 ^c
Fast Run	100	117.4 ^a	163.9 ^b	170.2 ^b
Glide	100	145.7 ^{a,b}	138.5 ^a	167.4 ^b
Pool	100	142.5 ^a	140.5 ^a	168.4 ^b
Plunge Pool	100	144.5 ^a	181.8 ^b	125.3 ^a
Rapid	100	144.0 ^{a,b}	140.9 ^a	166.6 ^b
Riffle	100	122.3 ^a	158.1 ^b	171.1 ^b
Run	100	125.4 ^a	168.7 ^b	157.3 ^b
Side Arm	100	142.9 ^a	149.8 ^a	158.8 ^a

^aClassifications with the same letter are not significantly different between flows ($\alpha=0.05$ level)

CONCLUSIONS

The overall goal of this project was to use a combination of HMU signatures, field techniques, and modeling to create a new overall methodology that would provide a scientific basis for understanding thresholds of change in biotic communities as a result of changes in streamflow. We sought to find a method to simplify data collection to visual estimates of hydraulic patterns. These patterns could then be used with habitat models to predict the probable composition of fish communities as well as other aquatic communities associated with these patterns.

Our statistical analyses show that various combinations of HMUs and flows follow predictable distributions. These distributions can be used in habitat models to predict fish communities. Field data collection efforts can be significantly reduced as a result (i.e. only enough data to identify the distribution is necessary). Further, combinations of HMUs and flows also show distinct signatures (as evidenced by the signature plots) which may be used to identify habitat present in specific rivers. The effects of instream flow changes on habitat can be modeled using these signature plots.

This project resulted in a proof of concept of the modeling technique. The appropriate publication is under preparation. The next step will be to apply the method to a broader number of streams and assess its applicability.

STUDENT INVOLVEMENT

Hampshire College

Five students have worked on this project as part of Phases I and II, 2 paid summer interns, and 3 students working on independent projects throughout the school-year. Their activities are detailed below.

Miira Wirth (Summer 2006 intern)

Over the summer 2006, Miira was involved in many different stages of this project, but mainly focused on data collection and preparation. She worked on the initial reconnaissance survey of Dunbar Brook, in which we installed temperature loggers at several locations and performed a basic assessment of potential electro-fishing sites. However, due to permitting complications we later abandoned this river and decided to electrofish and survey other more accessible rivers, as well as to mine our wealth of data previously collected from projects on other rivers. In several surveys of the Swift River and Fort River in Amherst, MA, she worked as a part of a team to electrofish and map several sites.

She used data previously collected on the Pomperaug River, CT, to help identify river-specific patterns of hydromorphologic (HMU) signatures. Her goal was to determine whether or not fish habitat corresponds to specific HMU signatures by comparing pristine conditions to full-buildout conditions. She prepared the data for this comparison for further analysis by another student of Hampshire College. This meant the preparation of datasheets using the MesoHABSIM method to determine the proportions of available habitat for several fish species on the Pomperaug River under pristine and full-buildout conditions, under several flow conditions.

Suzanne Carlson (Fall 2006 semester intern) – Project title: *'Stream Flow and Affected Fish Populations'*

Throughout the semester, Suzanne worked with Hydrosignature Project staff at Hampshire College and the University of Massachusetts to compile data and generate habitat suitability graphs for the Pomperaug and Quinebaug Rivers. She first completed a literature review to familiarize herself with the current literature. This review has been added to the literature database for the project. Her final report is currently in preparation.

Nicholas Newcomb (Spring 2007 semester intern) – Project title: *'Relating Hydraulic Patterns with Fish Communities'*

Nick's focus is the analysis of the hydrosignature patterns found in the project streams. He has also completed a literature review. Currently, he is using geographic information systems data to produce the hydrosignature plots needed for Phase II of the project. His final project and results will be completed by the end of the semester.

Nicholas Newcomb (Fall 2007 semester intern)

Nick continued to work on his project during the fall semester. He also compiled additional data for the Phase II modeling.

Paula Mouser (Summer 2007)

Paula used her background in statistics to perform the statistical analyses of the HMU data. She identified distributions for the HMUs and flows and compared the distributions. She performed multiple statistical tests including histogram analysis, cluster analysis, pairwise comparisons, etc.

Julie Groff (Spring 2008 semester intern)

Julie helped organize data and prepare data sets for modeling.

University of Massachusetts

Daniel Sisí Maestre, Eva Alfonzo Corzo, Manuel Moreno Ruiz-Poveda and Roberto Martínez Romero are four graduate students of forestry from Madrid Polytechnic in Spain who helped in data collection and analysis.

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Environmental Behaviors of Engineered Nanoparticles in Water

Basic Information

Title:	Environmental Behaviors of Engineered Nanoparticles in Water
Project Number:	2007MA73B
Start Date:	3/1/2007
End Date:	2/29/2008
Funding Source:	104B
Congressional District:	First
Research Category:	Water Quality
Focus Category:	Water Quality, Toxic Substances, Solute Transport
Descriptors:	None
Principal Investigators:	Baoshan Xing, Baoshan Xing

Publication

1. Wang, X.L., J.L. Lu and B. Xing, 2008. Sorption of organic contaminants by carbon nanotubes: Influence of adsorbed organic matter. *Environ. Sci. Technol.* (42)3207–3212
2. Iorio, M, B. Pan, R. Capasso and B. Xing, 2008. Sorption of phenanthrene by dissolved organic matter and its complex with aluminum oxide nanoparticles. *Environ. Pollut.* (in press).
3. Pan, B., D.H. Lin, H. Mashayekhi and B. Xing, 2008. Adsorption and hysteresis of bisphenol A and 17 α -ethinyl estradiol on carbon nanomaterials. *Environ. Sci. Technol.* (accepted).
4. Lin, D.H. and B. Xing, 2008. Phytotoxicity of zno nanoparticle: inhibition of ryegrass growth. Preprints of Environ. Chem. Div. Extended Abstracts of the 235th ACS national meetings. 48(1): 276–280
5. Mashayekhi, H., W. Jiang and B. Xing, 2008. Metal oxide nanoparticles show toxicity to bacteria. Preprints of Environ. Chem. Div. Extended Abstracts of the 235th ACS national meetings. 48(1): 326–328.
6. Pan, B. and B. Xing, 2008. Sorption of endocrine disrupting chemicals on carbon nano materials.” Preprints of Environ. Chem. Div. Extended Abstracts of the 235th ACS national meetings. 48(1): 206–208.
7. Wang, X.L. and B. Xing, 2008. Dissolved organic matter affects sorption of organic contaminants on carbon nanotubes. Preprints of Environ. Chem. Div. Extended Abstracts of the 235th ACS national meetings. 48(1): 220–223.
8. Wang, Z.Y., Z.J. Tian, F.M. Li, and B. Xing, 2008. Effects of five nanomaterials on gymnodinium breve. Preprints of Environ. Chem. Div. Extended Abstracts of the 235th ACS national meetings. 48(1): 315–319.

Problem and Research Objectives:

Knowledge of engineered nanoparticles in water is critical for evaluating their environmental fate, exposure, toxicity and risk. The two research objectives this year are:

- 1) To characterize the physical and chemical properties of nanoparticles and their dynamic aggregation behaviors under different aqueous conditions;
- 2) To examine the adsorption and desorption of toxic pollutants and DOM by nanoparticles;
- 3) To evaluate the toxicity of several nanoparticles.

Methodology:

Batch sorption techniques, liquid scintillation counting, HPLC detection, microbial tests, TEM and SEM examinations, seed germination tests.

Principal Findings and Significance:

Our preliminary data show that nanoparticles are toxic to tested microbes and plants; more toxic than their corresponding bulk materials. This is significant because these particles may pose risk to different ecosystems once released to the environments. Our data also showed that the nanoscaled aluminum oxides adsorbed more dissolved organic matter (DOM) and phenanthrene than the microscaled particles. In addition, different DOM samples could coat on carbon nanotubes (CNTs), some significantly reduced sorption of organic contaminants by CNTs, while others have minimal effect. These results are expected to help understand the interaction between CNTs and organic contaminants and environmental behavior of CNTs.

Publications and Conference Presentations:

Several Conference Platform presentations at the 235th ACS national meetings, April 6-10, 2008, New Orleans.

a. Articles in Refereed Scientific Journals

- Wang, X.L., J.L. Lu and B. Xing. 2008. Sorption of organic contaminants by carbon nanotubes: Influence of adsorbed organic matter. *Environ. Sci. Technol.* 42:3207-3212
- Iorio, M, B. Pan, R. Capasso and B. Xing. 2008. Sorption of phenanthrene by dissolved organic matter and its complex with aluminum oxide nanoparticles. *Environ. Pollut.* (in press).
- Pan, B., D.H. Lin, H. Mashayekhi and B. Xing. 2008. Adsorption and hysteresis of bisphenol A and 17 α -ethinyl estradiol on carbon nanomaterials. *Environ. Sci. Technol.* (accepted).

b. Book Chapter

none

c. Dissertations

none

d. Water Resources Research Institute Reports

none

e. Conference Proceedings

- Lin, D.H. and B. Xing. 2008. Phytotoxicity of zno nanoparticle: inhibition of ryegrass growth. Preprints of *Environ. Chem. Div. Extended Abstracts of the 235th ACS national meetings.* 48(1): 276-280
- Mashayekhi, H., W. Jiang and B. Xing. 2008. Metal oxide nanoparticles show toxicity to bacteria. Preprints of *Environ. Chem. Div. Extended Abstracts of the 235th ACS national meetings.* 48(1): 326-328.
- Pan, B. and B. Xing. 2008. Sorption of endocrine disrupting chemicals on carbon nano materials." Preprints of *Environ. Chem. Div. Extended Abstracts of the 235th ACS national meetings.* 48(1): 206-208.
- Wang, X.L. and B. Xing. 2008. Dissolved organic matter affects sorption of organic contaminants on carbon nanotubes. Preprints of *Environ. Chem. Div. Extended Abstracts of the 235th ACS national meetings.* 48(1): 220-223.
- Wang, Z.Y., Z.J. Tian, F.M. Li, and B. Xing. 2008. Effects of five nanomaterials on *gymnodinium breve*. Preprints of *Environ. Chem. Div. Extended Abstracts of the 235th ACS national meetings.* 48(1): 315-319.

f. Other Publications

none

Student Support

- Hamid Mashayekhi
- Ph.D.
- Department of Plant, Soil & Insect Sciences

Notable Achievements and Awards (Baoshan Xing)

A certificate of appreciation for organizing a special ACS symposium on “Environmental Fate, Behavior, and Toxicity of Manufactured Nanomaterials”, April 6-10, 2008, New Orleans.

References:

<http://www.umass.edu/loop/people/articles/74195.php>

Development of a standardized protocol for fish bioassays detecting estrogenic exposure

Basic Information

Title:	Development of a standardized protocol for fish bioassays detecting estrogenic exposure
Project Number:	2007MA74B
Start Date:	3/1/2007
End Date:	2/29/2008
Funding Source:	104B
Congressional District:	First
Research Category:	Biological Sciences
Focus Category:	Methods, Non Point Pollution, Wastewater
Descriptors:	None
Principal Investigators:	Kathleen Francis Arcaro, Lauren Taylor Moffatt

Publication

1. Moffatt Lauren, 2008, "The Development and Characterization of Fish Gene Expression Bioassays for Detecting Aquatic Endocrine Disruptors and Other Emerging Contaminants", Ph.D. Dissertation, Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, MA 01003, 261pp.
2. Moffatt Lauren and Kathleen Arcaro, 2007, "Quantitative Biomarkers of Estrogenic Exposure in Fish; Gene Expression Bioassays in two Model Species", Massachusetts WRRC 4th Annual Meeting (April 9th, 2007, Amherst, MA) and American Water Resources Association Specialty Conference on Emerging Contaminants (June 25th–27th, 2007, Vail, CO). (Poster)

Problem and Research Objectives:

Endocrine disrupting compounds are of increasing concern in waterways throughout the Northeast. Specifically, estrogenic contaminants have been detected downstream of agricultural operations, industrial discharges, and even municipal wastewater treatment facilities. Concern regarding the feminization of fish and wildlife species has led to the demand for sensitive, accurate, and reliable means to detect estrogenic activity in water samples. Fish models are ideal for examining potentially polluted waterways because fish are directly exposed to, and quickly concentrate aquatic contaminants. In concert with the use of molecular techniques, specifically the analysis of changes in gene expression, fish bioassays can be a sensitive indicator of estrogenic pollution. Although vitellogenin is a well-established biomarker of estrogenic endocrine disruption in male fish models, there is a great deal of variability in the assay design used by investigators measuring its induction. Despite the importance of vitellogenin as a biomarker, there has been no published study in which the parameters that affect vitellogenin expression, including length of exposure, number of fish per tank, volume of water per tank and frequency of water changes have been systematically investigated. Therefore, investigating the effects of experimental variables on bioassay outcome will help to characterize the assay with respect to its utility and limitations, and may aid in establishing a standard protocol resulting in a rapid and sensitive bioassay. The goal of this work was to characterize the sensitivity of the fish vitellogenin bioassay to changes in experimental design.

Methodology:

Male Japanese medaka (*Oryzias latipes*) were exposed in the laboratory to a range of environmentally detected concentrations of 17 β -estradiol (E₂) over a 96 hour time course. Water changes for all exposures occurred every 24 hours along with new chemical (or 1:10,000 or 1:1 million DMSO solvent control, depending on dilutions used to achieve exposure concentrations) addition to ensure constant exposures. At given time points, fish were anesthetized in buffered 0.5% tricaine methanesulfonate (MS-222) until immobile, blotted dry and weighed, and then decapitated immediately prior to dissection. Tissues removed (brain, liver, and gonad) from each fish (1 fish = 1 sample) were placed immediately into RNAlater solution and stored at 4°C until RNA isolation, or archived at -20°C for later use. High quality RNA was isolated from liver tissue using TRI-Reagent and quantified before being used in real time RT-PCR reactions along with gene-specific primers to quantify expression of vitellogenin and housekeeping gene mRNA. Vitellogenin quantities were normalized to the housekeeping gene and a combination of t-tests (p<0.05) and one way ANOVA (p<0.05) were used to determine statistically significant induction of vitellogenin over controls.

Experimental parameters that were manipulated (independently or in combination) in a series of experiments included the specific estrogenic compound (E₂, 17 α -ethynyl estradiol EE₂, or bisphenol A), the concentration of compound (1, 10, 100, or 1000pM), the length of exposures (24, 48, 72, or 96 hours), the volume of water per 2 fish used in exposure tanks (0.5L, 1.0L, or 2.0L), the lag/depuration time between chemical exposure and dissection/analysis (immediate, 24, 48, or 72 hours), and feeding status of fish (fed during exposure as in colony, versus unfed). These variables were examined with respect to their effects on vitellogenin mRNA induction.

Principal Findings and Significance:

The medaka vitellogenin mRNA bioassay was found to be sensitive and reliable in detecting levels of estrogens as low as 100pM (with environmentally detected levels being around 600pM), with the exception of bisphenol A which was undetectable via vitellogenin induction at the range of concentrations

tested. This is not entirely surprising considering the literature reports of bisphenol A's very weak estrogenic capabilities. Vitellogenin induction typically occurred in as little as 24 hours and was significant over controls at 48 hours. This assay is therefore rapid and sensitive. It was found that changing the volume of water in which the fish were exposed to E₂ did not affect the outcome of the assay in the range of volumes that we tested in this static system. However, it was determined that vitellogenin mRNA levels that have been induced by a short exposure to E₂ will begin to decline after 24 hours of depuration or absence of estrogenic contamination, and will continue to decrease over time. This indicates that samples should be processed in a rapid manner after exposure occurs. It was also found that, although not feeding the animals during exposure did not significantly reduce the vitellogenin response, the expression of some genes involved in energy balance and stress response were altered, indicating longer exposures and therefore deprivation of food could result in a change in vitellogenin response, or reproductive response entirely. Perhaps most importantly, it was found within the first set of experiments performed that the handling and presentation of gene expression data can alter the interpretation of the outcome. Specifically, data presented as fold change (obtained from dividing the relative expression values of treated animals by the expression values in control animals) did not typically resemble the data presented as relative quantities with both treatment and control displayed side by side. The reason for this is that although baseline, control levels of vitellogenin in male fish are all very low (hundreds to thousands fold lower than in animals treated with 100pM or higher of E₂), there is a substantial amount of individual animal variability at those low levels. This variability, which is likely biologically meaningless, can be by tens to almost hundreds fold, thus affecting the fold change data where the denominator becomes highly variable. Therefore, it was demonstrated that in this assay the more appropriate presentation of the data is through showing relative quantities of vitellogenin expression in both treated animals and control rather than fold change.

The work performed here has helped determine the sensitivity of the medaka vitellogenin mRNA assay to variability in experimental parameters. Both the flexibility and limitations of the assay have been characterized. This work has shown that the outcome of the assay is sensitive to some variables and not to others, however careful consideration must still be given to the design of such experiments because of the nature of the assay. In other words, the model is shown to be innately sensitive with respect to the accumulation of pollutants that occur in fish and the sensitivity of real time RT-PCR, making it a good sentinel of low levels of estrogenic contaminants, but a tool that must be handled appropriately to obtain meaningful and reliable results. Furthermore, the handling and interpretation of the data must be managed appropriately to gain the most biologically and environmentally relevant information.

References

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2. Orlando EF et al. 2004. Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow. *Environ Health Perspect.* 112(3): 353-358.
3. Sumpter JP, Jobling S. 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environ. Health Perspect.* 103:173-178.
4. Garcia-Reyero N et al. 2004. Use of vitellogenin mRNA as a biomarker for endocrine disruption in feral and cultured fish. *Anal. Bioanal. Chem.* 378:670-675.
5. Orn S et al. 2006. Comparison of Vitellogenin Induction, Sex Ratio, and Gonad Morphology Between Zebrafish and Japanese Medaka After Exposure to 17alpha-Ethinylestradiol and 17beta-Trenbolone. *Arch Environ Contam Toxicol.* Epub ahead of print.

6. Kang IJ et al. 2002. Effect of 17 β -estradiol on the reproduction of Japanese medaka (*Oryzias latipes*). *Chemosphere*. 47:71-80.
7. Yamaguchi A et al. 2005. Short-term effects of endocrine disrupting chemicals on the expression of estrogen-responsive genes in male medaka (*Oryzias latipes*). *Aquat. Toxicol.* 72:239-249.
8. Min et al. 2003. Effects of endocrine disrupting chemicals on distinct expression patterns of estrogen receptor, cytochrome P450 aromatase and p53 genes in *Oryzias latipes* liver. *J Biochem Mol Toxicol.* 17(5):272-7.

Information Transfer Program Introduction

None.

Water Resources Research Conference 2007

Basic Information

Title:	Water Resources Research Conference 2007
Project Number:	2007MA110B
Start Date:	3/1/2007
End Date:	2/29/2008
Funding Source:	104B
Congressional District:	1st District of MA
Research Category:	Not Applicable
Focus Category:	Water Quality, Hydrology, Non Point Pollution
Descriptors:	None
Principal Investigators:	Sarah Dorner, Marie–Francoise Walk

Publication

1. Walk, Marie–Françoise, 2008, Massachusetts Water Resources Research Center 5th Annual Conference: Integrating Water Resources Management – Final Program, April 8, 2008.
http://www.umass.edu/tei/wrrc/WRRC2004/pdf/Final%20Program_2008.pdf

The Water Resources Research Center organized the fifth annual Water Resources Research Conference: *Integrating Water Resources Management*.

Though the conference took place after this reporting period, on April 8, 2009, a great deal of work took place during this fiscal year to prepare the conference. The Cooperative State Research, Education, and Extension Service New England Regional Program again cooperated in planning the conference. Six co-sponsors helped underwrite the cost of the conference. Attendance increased from last year, to 144. Thanks to an increased sponsorship from the Massachusetts Department of Environmental Protection, attendance and presentations by DEP personnel were greatly increased this year. The Steering Committee was expanded to include many non-UMass professionals.

Thirty posters were presented and there were 36 paper platform presentations in three concurrent sessions. The presentations were grouped into four tracks subdivided into three sessions each:

Water Resources Management and Planning

- Water Quality and Enforcement
- Effective Water Management Regulations
- Water Resources Planning

Water Issues in the Field, Lab, and Classroom

- Fish: Water Resources Management Indicator
- Water Research and Climate Change
- Case Studies in Water Resources Education

Stormwater Challenges

- Low Impact Development
- Stormwater Best Management Practices
- Stormwater Monitoring and Management

Identification, Assessment, and Remediation

- Surface / Ground Water Interactions
- Contaminants in Water
- Wastewater Issues

The Keynote Address was given by Ira Leighton, Deputy Regional Administrator for USEPA New England on *New Developments in Stormwater Policy and Remediation*. Followup communications with Mr. Leighton have led to the draft of stormwater modeling proposals at the WRRC.

USGS Summer Intern Program

None.

Student Support					
Category	Section 104 Base Grant	Section 104 NCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	6	0	0	0	6
Masters	4	0	0	0	4
Ph.D.	2	1	0	0	3
Post-Doc.	0	0	0	0	0
Total	12	1	0	0	13

Notable Awards and Achievements

Dr. Baoshan Xing, PI for WRIP-funded "Environmental Behaviors of Engineered Nanoparticles in Water", was given a certificate of appreciation for organizing a special ACS symposium on "Environmental Fate, Behavior, and Toxicity of Manufactured Nanomaterials", April 6–10, 2008, New Orleans. see <http://www.umass.edu/loop/people/articles/74195.php>

Lauren Moffat, a PhD candidate in Animal Biotechnology and Biomedical Sciences, University of Massachusetts Amherst who received WRIP funding for "Development of a Standardized Protocol for Fish Bioassays Detecting Estrogenic Exposure" received the Best Poster Award at the Fourth Annual MA WRRC Conference Sustainable Waters in a Changing World: Research to Practice, April 9, 2007 in Amherst, MA for her poster entitled "Quantitative Biomarkers of Estrogenic Exposure in Fish: Gene Expression Bioassays in Two Model Species."

Publications from Prior Years

1. 2005MA47B ("Monitoring Estrogenic Hormones – Undesired Fish Contraceptives, and ") – Articles in Refereed Scientific Journals – Yuegang Zuo and Yuejuan Lin, 2007, Solvent effects on the silylation–gas chromatography–mass spectrometric determination of natural and synthetic estrogenic steroid hormones. *Chemosphere*, (69) 1175–1176.
2. 2005MA47B ("Monitoring Estrogenic Hormones – Undesired Fish Contraceptives, and ") – Other Publications – Yuegang Zuo, Kai Zhang and Yuejuan Lin, 2008, Microwave–Enhanced Silylation of Natural and Synthetic Estrogenic Steroids. 6th International Microwaves in Chemistry Conference, MIT, Cambridge, MA, May 13–16, 2008.
3. 2005MA47B ("Monitoring Estrogenic Hormones – Undesired Fish Contraceptives, and ") – Other Publications – Jinwen Guo and Yuegang Zuo, 2008, GC Determination of Phthalate esters in River, Marine, Rain and Snow Water. University of Massachusetts Dartmouth 14th Annual Sigma Xi Research Exhibit, April 29–30, 2008, North Dartmouth, MA.
4. 2005MA47B ("Monitoring Estrogenic Hormones – Undesired Fish Contraceptives, and ") – Other Publications – Yuejuan Lin and Yuegang Zuo, 2007, Ion–pair HPLC Determination of estrogens and their Conjugates in Water Samples. 7th Csaba Horvath Medal Award Symposium, April 19–20, 2007, Hartford Convention Center, Hartford, Connecticut.