


Spring 2016

Population projection and habitat preference modeling of the endangered James Spiny mussel (*pleurobema collina*)

Marisa Draper
James Madison University

Follow this and additional works at: <https://commons.lib.jmu.edu/honors201019>

 Part of the [Applied Statistics Commons](#), [Biology Commons](#), [Biostatistics Commons](#), [Environmental Health and Protection Commons](#), [Longitudinal Data Analysis and Time Series Commons](#), [Other Applied Mathematics Commons](#), [Other Mathematics Commons](#), and the [Population Biology Commons](#)

Recommended Citation

Draper, Marisa, "Population projection and habitat preference modeling of the endangered James Spiny mussel (*pleurobema collina*)" (2016). *Senior Honors Projects, 2010-current*. 231.
<https://commons.lib.jmu.edu/honors201019/231>

This Thesis is brought to you for free and open access by the Honors College at JMU Scholarly Commons. It has been accepted for inclusion in Senior Honors Projects, 2010-current by an authorized administrator of JMU Scholarly Commons. For more information, please contact dc_admin@jmu.edu.

Population Projection and Habitat Preference Modeling of the
Endangered James Spiny mussel (*Pleurobema collina*)

An Honors Program Project Presented to
the Faculty of the Undergraduate
College of Science and Mathematics
James Madison University

by Marisa Paige Draper

May 2016

Accepted by the faculty of the Departments of Mathematics and Statistics and Biology, James Madison University,
in partial fulfillment of the requirements for the Honors Program.

FACULTY COMMITTEE:

HONORS PROGRAM APPROVAL:

Project Advisor: Anthony Tongen, Ph.D.
Professor, Department of Mathematics and Statistics

Bradley R. Newcomer, Ph.D.,
Director, Honors Program

Reader: Lihua Chen, Ph.D.
Associate Professor, Department of Mathematics and
Statistics

Reader: Christine L. May, Ph.D.
Assistant Professor, Department of Biology

PUBLIC PRESENTATION

This work is accepted for presentation, in part or in full, at the Department of Mathematics and Statistics colloquium
on April 25, 2016.

Table of Contents

List of Figures	3
Acknowledgements	5
Abstract	6
Introduction	7
Methods	9
Typical Analysis Techniques	12
Mathematical Analysis - Matrix Population Modeling	12
Statistical Analysis - Odds of Detection	16
Mathematical Modeling	18
Dispersion Analysis	18
Hierarchical Clustering	22
Discussion	27
References	31

List of Figures

Figures

1	Visual detection of mussels using viewing scopes.	9
2	PIT-tag (left) and hallprint tag (right) attached to mussels with dental cement (Esposito, 2015).	10
3	Sample of the Excel spreadsheet for each data collection event including environmental data and date, time, GPS location, visual detection, quadrat (cell) number, grain size, data collectors, and notes for each mussel detected by the PIT-tag reader (Esposito, 2015).	11
4	Life cycle diagram for the James Spiny mussel and Notched Rainbow mussels, where G is the survivorship between stages, P is the survivorship within stages, and F is the fecundity.	12
5	Projection matrices for the James Spiny mussel (top) and Notched Rainbow using <i>Villosa lienosa</i> (middle) and <i>Villosa iris</i> (bottom) fecundity estimates.	14
6	Elasticity matrices for the James Spiny mussel and Notched Rainbow.	15
7	Sensitivity matrices for the James Spiny mussel and Notched Rainbow.	15
8	Dendrogram for the averaged location of James Spiny mussels for sweeps 5, 6, and 9.	22

9	Cluster definition for the James Spiny mussel for average locations in sweeps 5, 6, and 9 by distance in meters.	23
10	Cluster definition for the Notched Rainbow for average locations in sweeps 5, 6, and 9 by distance in meters.	23
11	Clumps and their centroids for the James Spiny mussel (top) and the Notched Rainbow (bottom). Dots represent individual mussels and stars represent clump centroids. Numbers indicate which row in Table 2 each clump corresponds to.	26

Tables

1	Species, sweep, number of mussels, VMR value, dispersion type, and critical threshold for a random classification for the average locations in 2014 (5, 6, 9 average) and four sweeps in 2015, including the averaged locations from the best three sweeps.	20
2	Average depth, velocity, and substrate of approximate 20 meter by 20 meter areas for 8 of the largest clusters of the James Spiny mussel and the Notched Rainbow.	25

Acknowledgements

This research was supported by the Thomas F. and Kate Miller Jeffress Memorial Trust. I would first like to thank the two other members of the mathematics team on this project, Maranda Pepe and Dylana Wilhelm, for working with me to obtain these results. I would like to extend a special thanks to Drs. Anthony Tongen, Lihua Chen, Patrice Ludwig, and Christine May, for this would not have been possible without their assistance and guidance. I am also grateful for the contributions of graduate students Alaina Epositio and Dorottya Boisen as well as undergraduate students Dakota Kobler and Katie Sipes and the spring 2015 Math/Biology 342 class. Finally, I would like to thank James Madison University's Mathematics and Statistics and Biology Departments for the continued support they provided throughout this project.

Abstract

The James Spinemussel (*Pleurobema collina*) is an endangered mussel species at the top of Virginia's conservation list. The James Spinemussel plays a critical role in the environment by filtering and cleaning stream water while providing shelter and food for macroinvertebrates; however, conservation efforts are complicated by the mussels' burrowing behavior, camouflage, and complex life cycle. The goals of the research conducted were to estimate detection probabilities that could be used to predict species presence and facilitate field work, and to track individually marked mussels to test for habitat preferences. Using existing literature and mark-recapture field data, these goals were accomplished by evaluating matrix population models, odds of detection based on environmental factors, dispersion type, and clustering trends. These results serve as the foundation of mathematical models used to aid in the recovery of the James Spinemussel and other cryptic species with sparse populations.

Introduction

Over a third of the world's species of mussels are found in North America. Of these 302 species, more than 70 percent are extinct or imperiled (US Geological Survey). According to the U.S. Fish and Wildlife Service, 15 out of 34 species within the genus *Pleurobema* are listed as endangered or threatened. The focus of this research, the James Spiny mussel (*Pleurobema collina*), is one of the many mussel species facing extinction. These organisms are crucial elements of their native ecosystems; in fact, a single mussel can filter several gallons of water each day. While siphoning water to gain nutrients, the mussels purify the water by removing algae, plankton, and other organic matter. This filtration also counters the negative effects of phosphorous and other potentially harmful compounds that can be detrimental to the stream. Despite being a vital contributor to environmental health, very little is known about the ecology of this immensely essential species.

The James Spiny mussel is critically endangered and one of the top species on Virginia's list for conservation; yet, there is currently no conservation action plan. Only one prior publication has addressed their life cycle (Hove and Neves, 1994). Analyses were also conducted on the Notched Rainbow (*Villosa constricta*), a similar species that is thriving in the same regions where the James Spiny mussel is fighting for survival. There is no evidence to suggest that the Notched Rainbow is competing with the James Spiny mussel since it is native to the area, there are abundant food sources, it uses different host fish for reproduction, and it has a disjoint reproductive season.

Both species are rare, cryptic, and have a complex life cycle. For successful fertilization to occur, female mussels must reside downstream from the males to siphon the sperm from the water. Once the eggs are fertilized, conglomerates consisting of hundreds of

mussel larvae are formed. The fecundity, or number of eggs per female, for the James Spinemussel is 12,423 on average, which is modest in comparison to many other mussel species (Hove and Neves, 1994; Haag, 2012). The female James Spinemussel releases the small, worm-like conglutinate from its shell just enough to lure one of the seven known host fish. In comparison, the Notched Rainbow uses a more accurate spraying mechanism to release its conglutinates. The host fish then ingests the conglutinate and the larvae attach to the gills to live parasitically until mature enough to drop off as juveniles. The juveniles burrow into the sediment at the bottom of the stream, where they remain until sexual maturation, which occurs at about three years for the James Spinemussel and one and a half years for the Notched Rainbow. The James Spinemussel's reproductive season is late May to early August, while the Notched Rainbow's season is August to June (Hove and Neves, 1994; Eads, Bogan, and Levine, 2006). The complex reproductive techniques of the James Spinemussel are likely a major contributor to the low probability of larval survival in comparison to other mussel species. The Notched Rainbow is more likely to be successful due to the method in which they release their conglutinates, age of sexual maturation, time frame for reproduction, and fecundity. Contrasting the James Spinemussel with the Notched Rainbow gives insight as to why one species is struggling while the other thrives under the same conditions. The research conducted seeks to track individually tagged mussels of both species in order to predict population trends, determine the conditions that increase the odds of detection, inform habitat preferences, and examine population clustering behaviors.

Methods

The field site in this study was a 230 meter stretch of Swift Run in Albemarle County, Virginia. In order to facilitate mussel sampling and tagging, the site was partitioned into quadrats, which are individual cells often used for the study of populations in ecology (Esposito, 2015). During the initial data collection event, known as a sweep, viewing scopes were used within the stream to visually detect mussels (Figure 1). When a mussel was found, a Passive Integrated Transponder tag (PIT-tag) was secured to its shell using dental cement. A distinct hallprint tag was also similarly attached to identify the mussel (Figure 2). The PIT-tag allowed an electronic reader, similar to a metal detector, to record the latitude and longitude coordinates of a tagged mussel when it passed over the mussel in a later sweep. If the PIT-tag reader indicated that a mussel was present, the researcher recorded whether or not the mussel was seen for mark-recapture analyses related to visual detection (Esposito, 2015). Additionally, the current stream conditions, such as temperature, turbidity, water height, and pH, were noted. There were ten sweeps recorded over the course of the summer and fall of 2014 and four in the spring and summer of 2015.



Figure 1. Visual detection of mussels using viewing scopes. (Photograph by C.L. May.)



Figure 2. PIT-tag (left) and hallprint tag (right) attached to mussels with dental cement (Esposito, 2015).

The GPS data collected from the PIT-tag reader were immediately synced to an online database after collection. These data were then organized into several Microsoft Excel spreadsheets so they could be easily accessed by Matlab (Figure 3). To compensate for error in the GPS coordinates recorded by the PIT-tag reader, only the most accurate data sets were used for analyses. Accuracy was determined by taking major weather events and cloud coverage into consideration, which could interfere with satellite communication. Within each data collection event, duplicate GPS coordinates for an individual mussel were averaged to further reduce error. The GPS coordinates from the three best data collection events in 2014 were also averaged, as mussel movement between these dates was expected to be minimal. This procedure could minimize some of the error of the PIT-tag reader in failing to detect mussels as well as error within GPS coordinates due to the surrounding environmental factors. The same procedure was followed for the four sweeps in 2015. Capture histories, which mark whether a mussel was detected and seen or not, were used with environmental variable data to calculate the odds of detection. The modified GPS coordinate data was used for dispersion and clustering analyses.

DATE	TIME	TAG TYPE	LATITUDE	LONGITUDE	TAG	EXPRESSION	CELL NUMBER	GRAIN SIZE	READER OPERATOR	SCOPE OPERATOR	NOTES
9/28/14	11:22:16	A0 TAG	38.201420 N	78.450333 W	*3DD.003BC88EF5	0	180-200	GS	AE	DB	
9/28/14	11:23:59	A0 TAG	38.201351 N	78.450203 W	*3DD.003BC88EF5	0	180-200	GS	AE	DB	
9/28/14	11:27:42	A0 TAG	38.201542 N	78.450172 W	*3DD.003BC88EC	0	180-200	S	AE	DB	
9/28/14	11:27:49	A0 TAG	38.201542 N	78.450211 W	*3DD.003BC88EE6	0	180-200	S	AE	DB	
9/28/14	11:28:06	A0 TAG	38.201530 N	78.450249 W	*3DD.003BC88EB7	0	180-200	S	AE	DB	
9/28/14	11:29:03	A0 TAG	38.201511 N	78.450211 W	*3DD.003BC88EC	0	180-200	S	AE	DB	
9/28/14	11:29:13	A0 TAG	38.201469 N	78.450180 W	*3DD.003BC88EB7	0	180-200	S	AE	DB	
9/28/14	11:32:13	A0 TAG	38.201450 N	78.450150 W	*3DD.003BC88ED4	0	180-200	PS	AE	DB	Under Hydrilla mat
9/28/14	11:32:54	A0 TAG	38.201431 N	78.450089 W	*3DD.003BC88EE6	0	180-200	GS	AE	DB	
9/28/14	11:35:11	A0 TAG	38.201389 N	78.450157 W	*3DD.003BC88EBD	0	180-200	S	AE	DB	
9/28/14	11:38:32	A0 TAG	38.201439 N	78.450188 W	*3DD.003BC88EC8	0	170-180	S	AE	DB	on waterline
9/28/14	11:39:48	A0 TAG	38.201519 N	78.450211 W	*3DD.003BC88EF0	0	170-180	S	AE	DB	near waterline

Figure 3. Sample of the Excel spreadsheet for each data collection event including environmental data and date, time, GPS location, visual detection, quadrat (cell) number, grain size, data collectors, and notes for each mussel detected by the PIT-tag reader (Esposito, 2015).

Typical Analysis Techniques

Mathematical Analysis - Matrix Population Modeling

Matrix population modeling was used to investigate the impact of each life stage on the extinction of the James Spiny mussel. The Notched Rainbow was also analyzed for comparative purposes. These models used survivorship and fecundity information from the mussels' life cycles to predict the future survival of the species and critical life stages for improvement (Figure 4). Estimates from literature were utilized to determine these survivorship and fecundity quantities (Hove and Neves, 1994; Haag, 2012; Asher and Christian, 2012). The model only took female mussels into consideration; thus, the fecundity was divided by two. This analysis was independent of the interaction of the mussels with the host fish.

Life Cycle Diagram

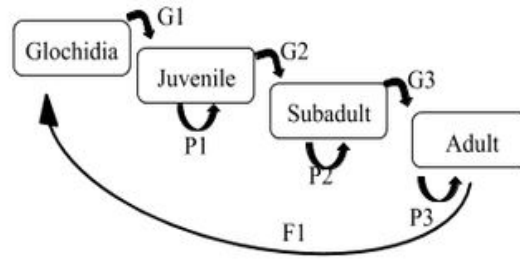


Figure 4. Life cycle diagram for the James Spiny mussel and Notched Rainbow mussels, where G is the survivorship between stages, P is the survivorship within stages, and F is the fecundity.

It is known that the average glochidia per female is 12,433 for the James Spiny mussel (Hove and Neves, 1994). The fecundity of the Notched Rainbow, *Villosa constricta*, is not known, so other *Villosa* species were used for this estimate. Within the genus *Villosa*, recent phylogenetic analysis has revealed that these species come from more than one ancestral family

(Asher and Christian, 2012). Consistent with this finding, the fecundity of *Villosa iris* is 27,849 and the fecundity of *Villosa lienosa* is 143,833 (Asher and Christian, 2012; Haag, 2012). To compensate for the dissimilarity within the genus, both species' fecundity values were used to estimate the Notched Rainbow's fecundity for matrix population analysis.

To calculate the probability of survival from egg to juvenile, the probability of fertilization, probability of fish infestation, and probability of successful juvenile drop off were considered. The percent of gravid females, mussels that are ready to release their glochidia, was calculated by using data collected from Craig, Johns, and South Fork Potts Creeks in 1988 and 1989 (Hove and Neves, 1994). For each year, the percent of gravid females was calculated by dividing the total number gravid by the number examined. The average percent of gravid females was 0.39. This value estimates the probability of fertilization, as gravidity only occurs in fertilized females. The average percent of fish infested per gravid female was calculated by dividing the percent of fish infested by the number of gravid females. This value, which can also be thought of as the probability of infestation for a gravid female, was calculated to be 0.002. The data for these calculations came from a study in South Fork Potts Creek, so they may vary slightly between populations due to population size and stream conditions (Hove and Neves, 1994). To calculate the average successful juvenile yield per infested host fish, the total number of juveniles collected was divided by the number of fish infested to obtain a value of 0.897. This value means that multiple fish must be infested to produce one successful juvenile. There are multiple other variables that could affect survival from glochidia to juvenile, including glochidia abortion and the probability of survival after drop-off until the time of burrowing. Considering these factors, combined with inherent extraneous variables, the estimate for survival from glochidia to juvenile is a best case scenario. Factoring in additional probabilities would further

reduce this value. A possible estimate was calculated by multiplying the probability of gravidity, probability of successful infestation, and the average juvenile yield per infested fish to obtain a value of 0.00075. Estimates for juvenile to subadult, subadult to adult, and within adult survivorship were 0.005, 0.844, and 0.844, respectively (Hove and Neves, 1994). For the Notched Rainbow, the probability of larval to juvenile survival was estimated to be 0.0075, which is 10 times higher than the James Spiny mussel, due to their more successful method of infestation. Survival probabilities for juvenile to subadult, subadult to adult, and within adult were also 0.005, 0.844, and 0.844, respectively, due to species similarities (Figure 5).

$$P_{James\ Spiny\ mussel} = \begin{bmatrix} 0 & 0 & 0 & 12423/2 \\ 0.0007546519 & 0 & 0 & 0 \\ 0 & 0.005 & 0 & 0 \\ 0 & 0 & 0.844 & 0.844 \end{bmatrix}$$

$$P_{Notched\ Rainbow\ (lienosa)} = \begin{bmatrix} 0 & 0 & 0 & 143833/2 \\ 0.007546519 & 0 & 0 & 0 \\ 0 & 0.005 & 0 & 0 \\ 0 & 0 & 0.844 & 0.844 \end{bmatrix}$$

$$P_{Notched\ Rainbow\ (iris)} = \begin{bmatrix} 0 & 0 & 0 & 27849/2 \\ 0.007546519 & 0 & 0 & 0 \\ 0 & 0.005 & 0 & 0 \\ 0 & 0 & 0.844 & 0.844 \end{bmatrix}$$

Figure 5. Projection matrices for the James Spiny mussel (top) and Notched Rainbow using *Villosa lienosa* (middle) and *Villosa iris* (bottom) fecundity estimates.

The elasticity and sensitivity of these matrices were calculated to determine which stages are most sensitive to constant and proportional change. The dominant eigenvalue for the James Spiny mussel was 0.873, which indicates that the population will reach extinction without conservation efforts. In contrast, the eigenvalue for the Notched Rainbow projection matrix was greater than one no matter which fecundity estimate was used, with values of 1.509 and 1.141 for

the *Villosa lienosa* and *Villosa iris* estimates, respectively. Therefore, the population is unlikely to go extinct. Based on the elasticity and sensitivity matrices for both mussel species, adult survivorship is most sensitive to proportional change (Figure 6). For the Notched Rainbow, survival from juvenile to subadult is most sensitive to constant change for both fecundity estimates (Figure 7). Survival from glochidia to juvenile is most sensitive to constant change for the James Spiny mussel (Figure 7). This implies that cultivation of James Spiny mussels in a facility to increase glochidia to juvenile survival and protection of adult mussels is the key to increasing species survival.

$$\begin{aligned}
 E_{James\ Spiny\ mussel} &= \begin{bmatrix} 0 & 0 & 0 & 0.030814 \\ 0.030814 & 0 & 0 & 0 \\ 0 & 0.030814 & 0 & 0 \\ 0 & 0 & 0.030814 & 0.876743 \end{bmatrix} \\
 E_{Notched\ Rainbow\ (lienosa)} &= \begin{bmatrix} 0 & 0 & 0 & 0.1898 \\ 0.1898 & 0 & 0 & 0 \\ 0 & 0.1898 & 0 & 0 \\ 0 & 0 & 0.1898 & 0.2407 \end{bmatrix} \\
 E_{Notched\ Rainbow\ (iris)} &= \begin{bmatrix} 0 & 0 & 0 & 0.1463 \\ 0.1463 & 0 & 0 & 0 \\ 0 & 0.1463 & 0 & 0 \\ 0 & 0 & 0.1463 & 0.4147 \end{bmatrix}
 \end{aligned}$$

Figure 6. Elasticity matrices for the James Spiny mussel and Notched Rainbow.

$$\begin{aligned}
 S_{James\ Spiny\ mussel} &= \begin{bmatrix} 0 & 0 & 0 & 0.000004 \\ 35.673870 & 0 & 0 & 0 \\ 0 & 5.384271 & 0 & 0 \\ 0 & 0 & 0.031897 & 0.907557 \end{bmatrix} \\
 S_{Notched\ Rainbow\ (lienosa)} &= \begin{bmatrix} 0 & 0 & 0 & 0.0000 \\ 37.9746 & 0 & 0 & 0 \\ 0 & 57.3152 & 0 & 0 \\ 0 & 0 & 0.3395 & 0.4305 \end{bmatrix} \\
 S_{Notched\ Rainbow\ (iris)} &= \begin{bmatrix} 0 & 0 & 0 & 0.0000 \\ 22.1419 & 0 & 0 & 0 \\ 0 & 33.4189 & 0 & 0 \\ 0 & 0 & 0.1980 & 0.5610 \end{bmatrix}
 \end{aligned}$$

Figure 7. Sensitivity matrices for the James Spiny mussel and Notched Rainbow.

Statistical Analysis - Odds of Detection

The odds of detection determine whether there are any factors that increase the likelihood of finding a mussel, which can help to expedite future field work. These analyses included predictors such as water height, mussel length, grain size, and season. The data were taken from a longitudinal study, meaning multiple observations were taken on each bivalve over time (Esposito, 2015). The response variable was expression, represented by 0 if not seen and 1 if visually detected. It is assumed that Y_{it} had a Bernoulli (π_{it}) distribution, where the indices $i=1, \dots, I$ and $t=1, \dots, T$ represent the bivalve and the sweep, respectively. The formula for expression is:

$$\log(\pi_{it}/(1-\pi_{it})) = \alpha + \beta_1 X_{it1} + \beta_2 X_{it2} + \dots + \beta_k X_{itk} + U_i$$

In this expression, U_i are independent random effects with a $N(0, \sigma^2)$ distribution and α and β_1, \dots, β_k are fixed effects. Y_{it} are conditionally independent given U_i . Since the standard deviation of the random effects, σ , was very small for the James Spinemussel, a simpler fixed effects model was used to analyze the detection of this species at a significance level of 0.15. Holding all other variables constant, the odds of detection of the James Spinemussel increase 10.6% (with a 95% confidence interval of -1.4% to 23.8%) with a 1 mm increase in mussel length, 2.1% (-0.7% to 4.9%) with a 1 mm increase in grain size, and decrease 88.7% in the fall (-98% to -10%) compared to the summer (Esposito, 2015). It is important to note that water height was not significant, as increased water height can bring more food and nutrients but could move the mussels downstream if too high, which could have important implications for the James Spinemussel's ability to survive and reproduce. Based on the results of the analyses, it

would be most efficient to look for the James Spiny mussel during the summer in areas with coarser grain sizes. The odds of detection of the Notched Rainbow, on the other hand, only increase by 16.7% (95%: 9.2% to 24.8%) with 1 mm increase in length. Grain size, season, and water height were not significant. This could indicate that the Notched Rainbow is more tolerant of a variety of habitats, which is supported by the species' success at the field site.

Mathematical Modeling

Dispersion Analysis

Dispersion, the spacing of individuals within a population, allows for a better understanding of the biological and ecological characteristics of a species. The three common patterns of dispersion are random, uniform, and clumped. In a random dispersion, the distance between individuals is unpredictable, which implies there is no competition or influence between members of the same species. A uniform dispersion suggests resource competition, as the distance between individuals is maximized to take advantage of the entire area. Finally, a clumped dispersion may indicate grouping for localized resources. Minimization of the distance between individuals may also imply social interaction or a group defense mechanism (Campbell, 2009).

The dispersion type of the James Spiny mussel and Notched Rainbow can be determined using the variance-to-mean ratio (VMR). Mean, M , is the average number of mussels in each quadrat while variance, V , indicates how widely the distance between mussels differs. The mean, variance, and variance-to-mean ratio are calculated using the following equations, where n is the number of quadrats studied and x_i is the number of individuals within each specific quadrat.

$$M = \bar{x} = \frac{1}{n} \sum_{i=1}^n x_i$$
$$V = s^2 = \frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2$$
$$VMR = \frac{V}{M}$$

A VMR less than one indicates a uniform dispersion, equal to one suggests a random dispersion, and greater than one implies a clumped dispersion. In addition to classifying dispersion type, the VMR can be used as an index for the degree of clustering. Thus, even though

the James Spiny mussel's dispersion is sometimes classified as clumped, it is much more weakly clumped than the Notched Rainbow. In order to accurately determine whether a dispersion was considered random, a critical threshold around 1 was determined by calculating 1,000,000 simulations of a random VMR. This was performed by taking into account the respective number of mussels and quadrats for each data collection event, or sweep. The simulated random VMR values for each sweep were subtracted from 1 in absolute value and the median was used as the critical threshold to create an interval around 1. When the variance to mean ratios were calculated for each individual sweep, the values were compared to this critical threshold to determine dispersion type. The PIT-tag reader often indicated multiple observations for the same mussel in a given sweep, so the duplicates of an individual mussel's GPS coordinates were averaged. For comparison, the locations of mussels for the three most accurate sweeps within 2014 and the four sweeps in 2015 were also averaged. The following table contains the VMR calculations for multiple sweeps of the Swift Run field site as well as the critical threshold used to determine whether a dispersion should be classified as random (Table 1).

Table 1. Species, sweep, number of mussels, VMR value, dispersion type, and critical threshold for a random classification for the average locations in 2014 (5, 6, 9 average) and four sweeps in 2015, including the averaged locations from the best three sweeps.

Species	Sweep	Number of Mussels	VMR	Dispersion Type	Critical Threshold
James Spiny mussel	5, 6, 9 average	20	1.3532	Clumped	[0.9297,1.0703]
James Spiny mussel	11	5	0.9649	Random	[0.9649,1.0351]
James Spiny mussel	12	18	1.0724	Random	[0.9276,1.0724]
James Spiny mussel	13	9	1.3845	Clumped	[0.9216,1.0784]
James Spiny mussel	14	9	1.1552	Clumped	[0.9310,1.0690]
James Spiny mussel	12, 13, 14 average	21	1.0071	Random	[0.9109,1.0891]
Notched Rainbow	5, 6, 9 average	53	2.0547	Clumped	[0.9123,1.0877]
Notched Rainbow	11	14	1.6953	Clumped	[0.8860,1.1140]
Notched Rainbow	12	36	1.8646	Clumped	[0.9117,1.0883]
Notched Rainbow	13	34	1.8645	Clumped	[0.9735,1.0265]
Notched Rainbow	14	23	1.2399	Clumped	[0.8997,1.1003]
Notched Rainbow	12, 13, 14 average	56	2.4732	Clumped	[0.9233,1.0767]

The dispersion of the James Spiny mussel alternates between random and clumped; however, the spacing often tends towards random. This random or weakly clumped dispersion of the James Spiny mussel may have negative implications for its reproductive success. Variation in dispersion type for the James Spiny mussel could also be partially explained by the small sample size. In comparison, the Notched Rainbow exhibited a clumped behavior across all sweeps. Since the Notched Rainbow is clumped, it is possible that they remain in close proximity for reproductive purposes. Due to the significantly larger sample size for the Notched Rainbow, the accuracy of this analysis is more precise. It is also possible that the dispersion of each mussel species depends heavily on the behavior of their respective host fish. The host fish for the James Spiny mussel and Notched Rainbow are disjoint. A common fish host for the James Spiny mussel tends to move around more sporadically, while a fish host specific to the Notched Rainbow often remains more inactive and is therefore more likely to drop multiple mussels in one area. It is

unknown how far mussels move laterally and if they tend to do so towards ideal habitats. Therefore, it may be more justifiable that another component of their environment, such as host fish or stream flow, may have a large impact on their dispersion.

The size of quadrats, and consequently the number of quadrats, greatly impacts the VMR calculations. In order to determine the optimal quadrat size to use, several different sizes were taken into consideration. This led to the formation of a theorem:

Theorem. *As the number of quadrats increases for any dispersion type,
the VMR will converge to one.*

As a result, it was determined that a large quadrat size should not be used to obtain an accurate variance to mean ratio. Appropriate sized quadrats should be determined based on the particular data being analyzed and, therefore, cannot be generalized for all data.

Hierarchical Clustering

Hierarchical clustering was used to visualize where the clusters of both species were occurring relative to substrate, velocity, and depth. Cluster size was defined by the distance sperm can travel while still successfully fertilizing the eggs of a female mussel downstream. This distance was expected to be tens of meters, so clusters were analyzed in ten meter increments (B. Ostby, 2015; J. Jones, 2015). A dendrogram connects mussels with a horizontal line at their respective distances (Figure 8). The groupings below the horizontal line drawn indicate the clumps defined at that distance. Similar to the VMR theorem, as the clump size is decreased, each mussel will be in its own clump, and as the clump size is increased, all mussels will be in the same clump.

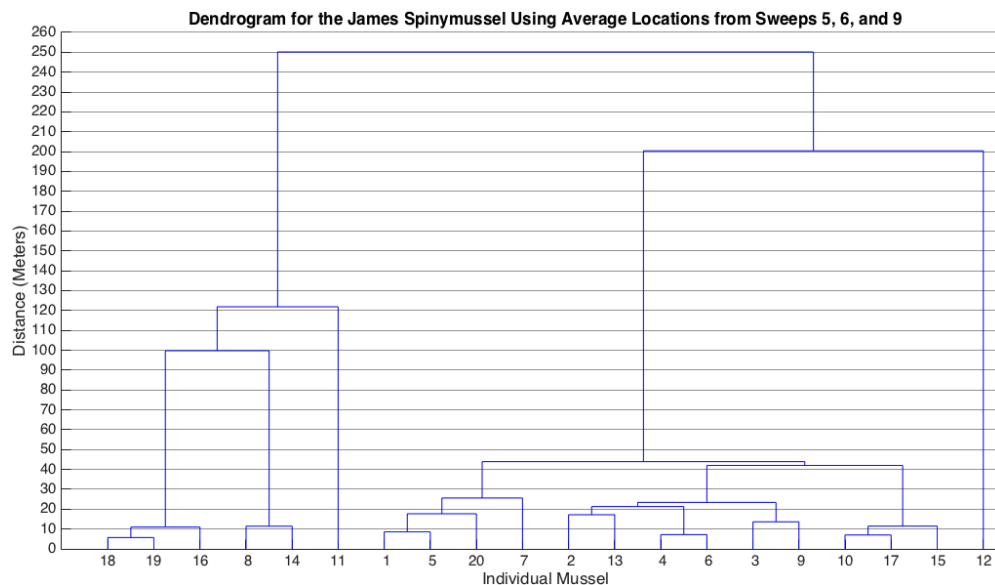


Figure 8. Dendrogram for the averaged location of James Spiny mussels for sweeps 5, 6, and 9.

The GPS data from the PIT-tag reader can be inaccurate due to substrate type, cloud cover, and other environmental factors interfering with the satellite signal. In order to minimize

this error, repeated data points for the same mussel were averaged for sweeps 5, 6, and 9. The locations for both the James Spiny mussel and the Notched Rainbow were plotted on a GoogleEarth® image of the stream in order to visualize where the clusters were occurring, with each colored oval representing a cluster (Figures 8, 9 & 10). Since streams often change a great deal from year to year, this analysis was only valid for the 2014 data collection events, the same year that the stream data were collected (Esposito, 2015). Although the James Spiny mussel had a much smaller sample size, the largest cluster for both species can be seen upstream. This area correlates to a riffle, an area blocked by a fallen tree that has shallow waters, slow velocity, and coarser grain sizes.

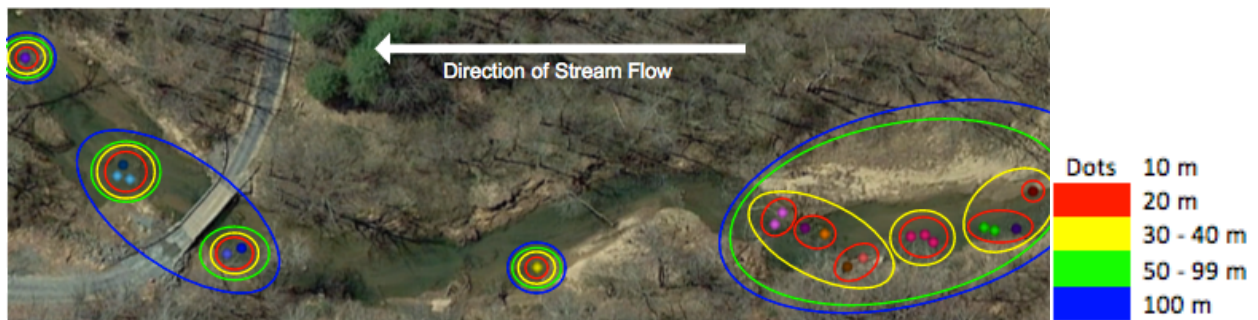


Figure 9. Cluster definition for the James Spiny mussel for average locations in sweeps 5, 6, and 9 by distance in meters.

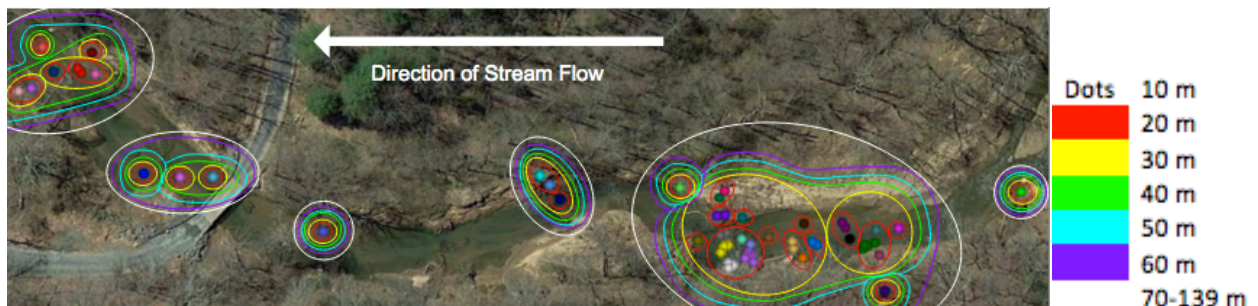


Figure 10. Cluster definition for the Notched Rainbow for average locations in sweeps 5, 6, and 9 by distance in meters.

Clusters determined by natural break-off points in the data can be found by analyzing the consistency of the dendrogram links. The height of each vertical line can be compared to the surrounding heights to detect significant changes in distance between points. Links that have similar heights will have small inconsistency values. Natural breaks in the data often occur when there is a large increase in the inconsistency value of the next grouping. A large inconsistency value indicates that the points of the new cluster are much further apart than the points in the previous cluster. This can be verified visually with the dendrogram. For the James Spiny mussel, the first inconsistency value larger than the others occurred just before the 50 meter line. The dendrogram shows a large increase in link length at this point, as the clusters remain constant until 100 meters (Figure 8). This is represented in the GoogleEarth® plot with green ovals (Figure 9). However, it is estimated that mussels must be within tens of meters in order for successful fertilization to occur (B. Ostby, 2015; J. Jones, 2015). Thus, it is problematic to draw a conclusion based on inconsistency values alone. Taking this constraint into consideration, habitat conditions were averaged for clumps defined at 20 meters.

In order to quantify trends for the areas in which the largest clumps were occurring for both species, the GPS coordinates of each mussel within a clump were averaged to obtain a centroid. An approximate 20 meter by 20 meter area was defined around the centroid to estimate average conditions for mussels in the area. This area and the corresponding values are approximate due to human error in data collection within the stream as well as naturally occurring changes in stream conditions. Depth, velocity, and substrate values for each one meter by one meter square within the range were averaged for the three largest clumps of the James Spiny mussel and the five largest clumps of the Notched Rainbow (Table 2). Table 2 is arranged by the location of the clump centroid (Figure 11). There is no significant difference in depth,

velocity, or substrate for the clumps of the James Spiny mussel and the Notched Rainbow. The depth ranges from 0.7 to 1.1 meters and the velocity ranges from 0.22 to 0.7 meters per second. Both species are found primarily in pebble sand or gravel, with only a few clumps having sand in their 20 meter by 20 meter blocks. Both clumps with sand as the primary substrate are located downstream in habitats that are considered transient. This analysis confirms that the ideal habitat for both species is the upstream riffle, where the water is deep, the current is slow, and the substrate is coarse.

Table 2. Average depth, velocity, and substrate of approximate 20 meter by 20 meter areas for 8 of the largest clusters of the James Spiny mussel and the Notched Rainbow.

Species	Number of Mussels	Depth (m)	Velocity (m/s)	Substrate (d90)
James Spiny mussel (1)	3	0.7	0.7	Pebble Sand/Gravel
James Spiny mussel (2)	3	1.1	0.22	Pebble Sand/Gravel
James Spiny mussel (3)	3	0.9	0.24	Sand
Notched Rainbow (1)	6	0.8	0.5	Pebble Sand/Gravel
Notched Rainbow (2)	3	1.1	0.33	Pebble Sand/Gravel
Notched Rainbow (3)	3	1	0.32	Sand/Pebble Sand
Notched Rainbow (4)	15	0.9	0.42	Pebble Sand
Notched Rainbow (5)	3	0.9	0.29	Sand



Figure 11. Clumps and their centroids for the James Spynymussel (top) and the Notched Rainbow (bottom). Dots represent individual mussels and stars represent clump centroids.

Numbers indicate which row in Table 2 each clump corresponds to.

Discussion

When faced with a sparse data set, potential analyses are limited to preserve statistical accuracy of the results. Typically, population projections are made using matrix population analysis, which also gives insight into the life stages of greatest concern through elasticity and sensitivity analysis. These life stages should be the focus of funding and conservation efforts. Another common method used to determine population trends is the odds of detection analysis. These results can facilitate future field work and make a preliminary estimate of habitat preferences based on the conditions in which the species is most often visually detected. Since spatial orientation is key to the reproductive success of bivalve species, GPS location data was used for dispersion and hierarchical clustering analyses. Dispersion analysis, a common method used in ecology, is used to quantify the degree of spacing between individuals in a population. The variance to mean ratio (VMR) is used to determine whether a species is mostly clumped together, randomly distributed, or uniformly spaced. The VMR is of interest for species that require proximity for population perseverance as well as species that must be more spaced out due to competition or other concerns. The next step in the spatial analysis process was hierarchical clustering, which allowed the visualization of groupings determined by dispersion analysis. Hierarchical clustering shows where the largest and smallest clusters occur in the field site, which can facilitate future field work and give insight into habitat preference models based on the average conditions in an area that is more heavily populated by the species. These additional techniques can be helpful when looking at a small population with sparse data. The key elements of survival, odds of detection, and spatial arrangement play a large part in determining why a species is struggling, especially when compared to a similar, more abundant species.

Within this data set, matrix population analysis and odds of detection analysis allow for population projections and some insight into species behavior based on where they are seen with the highest frequency. Sensitivity and elasticity analysis determine the life stage of greatest concern for conservation efforts. For the James Spiny mussel, proportional increases in glochidia to juvenile and adult survival contribute the most towards greater species survival. For the Notched Rainbow, juvenile to subadult and adult survival have the greatest impact. For both species, adult survival is most sensitive to constant change, an assumption that may extend to other bivalve species. This result suggests that protection of existing populations and cultivation of juvenile James Spiny mussels in a facility are the most effective methods to increase survival. Additionally, the odds of detection infer population behavioral trends and facilitate field work by emphasizing specific habitat types. The odds of detection for the James Spiny mussel increase as mussel length and grain size increase and decrease in the fall, which suggests it is best to find James Spiny mussels in the summer in larger grain sizes for future tagging events. The odds of detection for the Notched Rainbow only increase as length increases, implying that this species is much more tolerant of a variety of habitat types, which likely contributes to its greater success.

Further techniques included dispersion and cluster analyses. Cluster analysis is frequently used to identify similarities and differences between individuals, but has not been used for habitat preference modeling of bivalves. When presented with small to moderately sized data sets, these methods can give more information about behavioral patterns, spatial arrangement, and habitat preferences. The GPS coordinates obtained in each data collection event were plotted overlaying a GoogleEarth map of the field site to determine the variance-to-mean ratio. This value determines whether a population has a tendency to be clumped, randomly spaced, or uniformly spaced on a continuous scale. Quadrat size is key for this analysis and

should be correlated with species and field site size. The Notched Rainbow always had a clumped dispersion, while the James Spinemussel tended to be weakly clumped or random. This result has negative implications for their reproductive cycle as mussels must be within tens of meters for successful fertilization to occur. The dispersion of the Notched Rainbow also implies that there is not competition between conspecifics since this species is thriving in a clumped pattern. To visualize where these clumps were occurring and the number of mussels in each clump, hierarchical clustering was analyzed in ten meter increments. The largest clump for both species is found upstream in what is thought to be a very stable environment due to woody debris, coarse grain sizes, and slower velocities, known as a riffle. It is estimated that mussels must be within tens of meters for successful fertilization to occur, so further calculations were carried out to determine average depth, velocity, and substrate for each clump defined at 20 meters. Preliminary analysis using cluster centroids to quantify habitat trends confirmed that the most likely habitat for clumps of both species has an average water depth of 0.7 to 1.1 meters, an average velocity of 0.22 to 0.7 meters per second, and is most often pebble-sand or gravel, both coarse substrate types. This can be used as a preliminary habitat preference model for the detection and release of the James Spinemussel and Notched Rainbow. This method, while frequently used to identify similarities and differences between individuals, but has not been used to identify clusters for habitat preference models of bivalves.

If no action is taken, the James Spinemussel will likely go extinct while the Notched Rainbow will continue to thrive according to matrix population analysis. In order to preserve this species, it would be most effective to cultivate juveniles in a lab setting for greater glochidia survival. Protective measures should be taken to increase the survival probability of adult mussels as well. The Notched Rainbow's clumped dispersion aids in its reproductive success in

combination with their spraying method for host fish infestation. Releasing cultivated James Spiny mussels in a similar clumped pattern would help increase fertilization to account for the low probability of infestation. In the sampled field site, the mussels should be released upstream with the existing successful colony. Other stream sites should be analyzed for habitat compatibility and success to determine the most advantageous juvenile release location. The odds of detection and cluster analyses both imply that James Spiny mussels show colony persistence in coarser grain sizes where there is also slow, deep water. It may also be helpful to shield mussels from potential predation with debris, as the most populous area also contains a fallen tree. By informing this action plan, the inevitable extinction of the James Spiny mussel can potentially be averted. Other bivalve species facing extinction may also benefit from similar analyses to determine the best course of action for conservation. The preservation of these organisms that are so vital to the ecosystem is key to avoid long-term devastating environmental effects. These analyses serve as a foundation to inform their recovery.

References

- Asher, A. M., & Christian, A. D. (2012). Population Characteristics of the Mussel *Villosa iris* (Lea) (Rainbow Shell) in the Spring River Watershed, Arkansas. *Southeastern Naturalist*, 11(2), 219-238.
- Campbell, N. (2009). Population Ecology. In *Biology: Concepts & connections* (6th ed.). San Francisco: Pearson/Benjamin Cummings.
- Eads, C. B., Bogan, A. E., & Levine, J. F. (2006). Status and Life-history Aspects of *Villosa constricta* (Conrad 1838) (Notched Rainbow), in the Upper Neuse River Basin, North Carolina. *Southeastern Naturalist*, 5(4), 649-660.
- Esposito, Alaina C., "Using Capture-Mark-Recapture Techniques to Estimate Detection Probabilities & Fidelity of Expression for The Critically Endangered James Spinemussel (*Pleurobema collina*).\" (2015). Masters Theses. Paper 66.
- Galbraith, H., Maloney, K., Hamilton, H., & Puckett, C. (2013, April 04). The Secret Lives of Mussels: America's Most Endangered Species! Retrieved from http://www.usgs.gov/blogs/features/usgs_top_story/the-secret-lives-of-mussels-america-s-most-endangered-species/
- Haag, W. (2012). *North American freshwater mussels: Natural history, ecology, and conservation*. Cambridge [England]: Cambridge University Press.
- Hove, M. and Neves, R. (1994). Life history of the endangered James Spinemussel (*Pleurobema collina*) (Conrad, 1837) (mollusca: Unionidae). 11(1):29-40.
- Jones, J. (2015). Personal interview.
- Moser, G. A. (1990). James Spinemussel (*Pleurobema collina*) recovery plan. U.S. Fish and Wildlife Service.

Ostby, B. (2015). Telephone interview.

US Fish and Wildlife Service ECOS Environmental Conservation Online System. Retrieved February 08, 2016, from http://ecos.fws.gov/tess_public/reports/ad-hoc-species-report?kingdom=V