2016

POPULATION GENETIC STRUCTURE OF NECTURUS MACULOSUS IN CENTRAL AND EASTERN KENTUCKY

Mason Owen Murphy

University of Kentucky, masonomurphy@gmail.com
Digital Object Identifier: http://dx.doi.org/10.13023/ETD.2016.093

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Recommended Citation

Murphy, Mason Owen, "POPULATION GENETIC STRUCTURE OF NECTURUS MACULOSUS IN CENTRAL AND EASTERN KENTUCKY" (2016). Theses and Dissertations--Biology. 33.
https://uknowledge.uky.edu/biology_etds/33

This Master's Thesis is brought to you for free and open access by the Biology at UKnowledge. It has been accepted for inclusion in Theses and Dissertations--Biology by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student’s advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student’s thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Mason Owen Murphy, Student

Dr. David W. Weisrock, Major Professor

Dr. David F. Westneat, Director of Graduate Studies
POPULATION GENETIC STRUCTURE OF *NECTURUS MACULOSUS* IN CENTRAL AND EASTERN KENTUCKY

THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in the College of Arts and Sciences at the University of Kentucky

By

Mason Owen Murphy

Lexington, Kentucky

Co-Directors: Dr. David W. Weisrock, Associate Professor of Biology

and Dr. Steven J. Price, Assistant Professor or Stream and Riparian Ecology

Lexington, Kentucky

2016

Copyright © Mason Murphy 2016
ABSTRACT OF THESES

POPULATION GENETIC STRUCTURE OF *NECTURUS MACULOSUS* IN CENTRAL AND EASTERN KENTUCKY

Population structure is influenced by extrinsic factors, such as landscape architecture and dispersal barriers. Lotic network architecture is known to constrain ecological, demographic and evolutionary processes, including population genetic structure. I assessed the population structure of a widespread aquatic salamander, *Necturus maculosus*, across three river basins in central and eastern Kentucky. I examined the role of network architecture, anthropogenic barriers, and spatial scale on patterns of population structure. I also provided a review of *N. maculosus* capture methods and offer an improved trap design. I identified significant structuring between the combined Licking/Kinniconick basin and the Kentucky River basin, with further structure within each basin. I found evidence for both hierarchically organized populations structure (e.g. Stream Hierarchy Model), as well as population structure unaffected by network hierarchy (e.g. Death Valley Model). These results highlight the importance of scale when examining population structure. Whereas one model may suffice to explain population structure at a local scale, a second model may be necessary to accurately describe the population structure across larger spatial scales. These results suggest that local factors affect population structure uniquely across a species’ range, and support a multi-model approach for assessing population structure.

KEYWORDS: lotic networks, *Necturus maculosus*, population genetic structure

Mason Owen Murphy

April 27, 2016
POPULATION GENETIC STRUCTURE OF *NECTURUS MACULOSUS* IN CENTRAL AND EASTERN KENTUCKY

By

Mason Owen Murphy

David W. Weisrock, Ph.D.
Co-Director of Thesis

Steven J. Price, Ph.D.
Co-Director of Thesis

David F. Westneat, Ph.D.
Director of Graduate Studies

April 27, 2016
ACKNOWLEDGEMENT

I would like to acknowledge the support of my family and friends, especially my parents, whose unwavering support was cherished. I would also like to thank my fiancée Rachel, whose love and belief in me helped me through the entire process.

I thank my committee member, Dr. Catherine Linnen, who offered lab, technical, and methodological support. I would especially like to thank my advisors, David Weisrock and Steven Price, whose guidance has helped me fully appreciate conducting good science.

I greatly appreciate the University of Kentucky Department of Biology for funding my graduate education.

Lastly, I would like to thank all of the organizations that provided funding for this project, including: The Kentucky Science and Engineering Foundation, the Kentucky Academy of Sciences, the Society for Freshwater Science, the Kentucky Society for Natural History, and the University Of Kentucky Department of Biology, and the University of Kentucky Department of Forestry.
# TABLE OF CONTENTS

Acknowledgement ........................................................................................................... iii

List of Tables ....................................................................................................................... v

List of Figures ..................................................................................................................... vi

Chapter One: A review of common mudpuppy (*Necturus maculosus*) capture methods and a description of a revised trap design

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Review of Capture Methods</td>
<td>2</td>
</tr>
<tr>
<td>Electroshocking</td>
<td>2</td>
</tr>
<tr>
<td>Manual surveys</td>
<td>3</td>
</tr>
<tr>
<td>Seining</td>
<td>3</td>
</tr>
<tr>
<td>Modified minnow traps</td>
<td>4</td>
</tr>
<tr>
<td>Other methods</td>
<td>4</td>
</tr>
<tr>
<td>New trap design</td>
<td>5</td>
</tr>
<tr>
<td>Conclusions</td>
<td>7</td>
</tr>
</tbody>
</table>

Chapter Two: Population structure in a permanently aquatic salamander across multiple spatial scales: the role of barriers and river structure

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>14</td>
</tr>
<tr>
<td>Methods</td>
<td>19</td>
</tr>
<tr>
<td>Sampling sites and design</td>
<td>19</td>
</tr>
<tr>
<td>Genetic data collection</td>
<td>20</td>
</tr>
<tr>
<td>Estimating genetic diversity</td>
<td>22</td>
</tr>
<tr>
<td>Assessment of population structure</td>
<td>22</td>
</tr>
<tr>
<td>Results</td>
<td>24</td>
</tr>
<tr>
<td>Discussion</td>
<td>26</td>
</tr>
<tr>
<td>Stream Hierarchy Model</td>
<td>27</td>
</tr>
<tr>
<td>Death Valley Model</td>
<td>29</td>
</tr>
<tr>
<td>Influence of scale on multiple models</td>
<td>30</td>
</tr>
<tr>
<td>Other Demographic Factors</td>
<td>32</td>
</tr>
<tr>
<td>Conclusions</td>
<td>33</td>
</tr>
</tbody>
</table>

Appendix ....................................................................................................................... 45

References ...................................................................................................................... 46

Vita ................................................................................................................................. 53
LIST OF TABLES

Table 1.1. Summary of previous common mudpuppy (*Necturus maculosus*) capture events............................................................9

Table 1.2. Summary of our common mudpuppy (*Necturus maculosus*) sampling for both manual surveys and trapping surveys using modified Briggler traps.........................10

Table 2.1. Sites sampled with corresponding site number, basin location, individuals captured, and GPS coordinates.................................................................34

Table 2.2. Geographic distance (km) matrix between sampling sites..........................35

Table 2.3. Total number of dams separating sampling sites.................................36

Table 2.4. Summary statistics for all ten sampling sites........................................37

Table 2.5. Pairwise *F*<sub>ST</sub> for all ten sampling sites........................................38

Table 2.6. Tajima’s D at multiple scales including sites, basins, population clusters, and all together.................................................................39

Table 2.7. AMOVA results for A) Populations according to Basin (3 Populations) and B) Populations according to Likelihood analyses (2 Populations).................................40
LIST OF FIGURES

Figure 1.1. Materials needed per trap ................................................................. 11

Figure 1.2. Modified Briggler trap ................................................................. 12

Figure 1.3. Mudpuppy captured in trap near Cynthiana Kentucky, USA............ 13

Figure 2.1. Location of sampling sites (triangles) and dams (black circles) in Kentucky... 41

Figure 2.2. Plot of Cross-validation errors for ADMIXTURE K selection............... 42

Figure 2.3. Bar plot with the admixture estimates for A) K = 2, B) K = 3, C) K = 4, and D) K = 5 ................................................................. 43

Figure 2.4. Principal components plot for two populations found using the find.clusters function in adegenet using Discriminant Function 1 on the x-axis ............... 44
CHAPTER ONE

A REVIEW OF COMMON MUDPUPPY (NECTURUS MACULOSUS) CAPTURE METHODS AND A DESCRIPTION OF A REVISED TRAP DESIGN

Introduction

Necturus maculosus is a widespread, aquatic salamander native to both lentic and lotic systems in eastern North America (Petranka 1998). These salamanders typically occur under cover such as large flat rocks or logs, especially in areas with layers of mud substrate and debris (Matson 2005; Petranka 1998). Adults often exhibit high site fidelity (Matson 1998; Shoop and Gunning 1967). Necturus maculosus has a long lifespan (~30 years; Bonin et al. 1995), and plays an integral role in its environment as a predator, feeding on fish, crayfish, and mollusks (Vandevalk and Coleman 2010). Breeding occurs in the fall; females store sperm in spermatheca over the winter with ovulation and fertilization delayed until spring (Matson 2005; Petranka 1998). Egg deposition occurs under large flat rocks in the spring and summer (Matson 2005; Petranka 1998). Larvae hatch in early summer, and there is evidence that adult N. maculosus attend and guard clutches of eggs (Hime et al. 2014). Additionally, N. maculosus is the only known host for the salamander mussel (Simpsonaias ambiguа), a regionally imperiled freshwater mussel.

While presumably common throughout its range (Barbour 1971; Petranka 1998), much of the life history of N. maculosus is unknown. For example, habitat preferences, seasonal movements, population structure, gene flow and dispersal are poorly understood (but see McDaniel et al. 2009). The lack of information is due, in part, to its cryptic nature and capture difficulty (Matson 1990). Here we review various capture methods for
*N. maculosus*, as well as illustrate and highlight a new trap design for their efficient capture.

**Review of Capture Methods**

A number of common methods are used for *N. maculosus* sampling, including electroshocking, manual surveys, seining, and trapping using minnow traps (Table 1.1).

*Electroshocking*

Electroshocking uses a mild electric current to stun aquatic vertebrates for easy capture with nets. While electroshocking has been used to successfully capture *N. maculosus* (Schmidt et al. 2004; Shoop and Gunning 1967; Vandevalk and Coleman 2010), it has numerous drawbacks, and may be ineffective (Matson 1990). Backpack electroshocking is limited by navigability and depth of the water, and is typically feasible in water where the sampler is able to wear waders (< 1 m deep). Boat-mounted electroshocking enables the sampling of larger systems, but limits smaller stream sampling and is cost prohibitive. Drawbacks of both electroshocking methods include dependency on adequate water conductivity to deliver the shock, known as a limited shock radius. Furthermore, *N. maculosus* tend to stay under large flat rocks, reducing the chance of netting a shocked *N. maculosus*, as the rock prevents the mudpuppy from rising to the surface (Matson 1990). Nickerson et al. (2002) and Nickerson and Krysko (2003) discourage the usage of electroshocking, given the possible non-target and negative effects on hellbender (*Cryptobranchus alleganiensis*) larvae. These concerns may apply to *N. maculosus* larvae as well.
Manual surveying

Manual surveying, by wading or skin diving, is also commonly used to sample for *N. maculosus*, especially in shallow water (Nickerson et al. 2002). This method involves walking or floating upstream while flipping large flat rocks typically used by *N. maculosus* for refuge. Benefits of this method include the opportunity to directly observe mudpuppies in their habitat, as well as a relatively high level of capture efficiency (Matson 1990). Drawbacks to this method include a dependency on low, clear water conditions, wadeable study sites, and an inability to sample deep water pools. Furthermore, when utilizing this method, skill is needed to hand capture or net each *N. maculosus*. Given the wide range of *N. maculosus* habitats, this method has had variable results, with better results in smaller lotic areas and shallow lentic areas (Gibbons and Nelson Jr. 1968; Matson 1990; Trauth et al. 2007).

Seining

Seining typically involves dragging a seine net through a river or stream, with at least one person disturbing debris and rock piles ahead of the seine, in order to remove mudpuppies from their habitat on the bottom of streams. Cagle (1954) found little success capturing adult *N. maculosus* using seines, however Matson (1990) found seining to be the most successful of four techniques tried. Seining seems to work best for capturing larval and immature *N. maculosus*, especially in streams where primary refugia is leaf litter, rather than large flat rocks (Cagle 1954; Matson 1990).
**Modified minnow traps**

Modified minnow traps have been the most utilized form of *N. maculosus* trapping in the last 50 years (Chellman and Parrish 2010; McDaniel et al. 2009). This method uses a standard minnow trap that has enlarged openings to allow for *N. maculosus* entry. These traps are typically baited with chicken liver, cat food, or raw fish (Gendron et al. 1999; Trauth et al. 2007), and are placed near perceived *N. maculosus* refugia in streams. Benefits of these traps include the ability to sample in deep and turbid water, as well as the ability to sample in freezing conditions without undue risk for hypothermia. Disadvantages to this capture method include low trap success at zero to 0.02 *N. maculosus* per trap night (Chellman and Parrish 2010; Matson 1990; McDaniel et al. 2009; Palis 2010; Trauth et al. 2007). Given low trap rates associated with this method, the usage of modified minnow traps is best executed when a large number of trap nights can be implemented, as few trap nights may result in no *N. maculosus* captures (Palis 2010; Trauth et al. 2007).

**Other methods**

Other less commonly used methods include fish trapnets and set lines (Bonin et al. 1995; Shoop and Gunning 1967; Vandevalk and Coleman 2010). Trapnets have not been frequently used in the last 50 years, but were used with minimal success in capturing *N. maculosus louisianensis* in Louisiana in the 1950’s, though recently Vandevalk and Coleman (2010) obtained *N. maculosus* captured incidentally in trap nets for their analyses. While baited trot lines had a similarly poor success rate (Cagle 1954), the use of set lines has been more successful (Cagle 1954; Shoop and Gunning 1967).
These two methods are characterized by baited hooks tied to trees or the shoreline, and are either floated (trot line) or not floated (set line). These methods have seen less use primarily due to a bias toward large juveniles and adults, as well as increased mortality rates from hook swallowing (Cagle 1954; Matson 1990; Shoop and Gunning 1967). Similar to the use of set lines, Bonin et al. (1995) was able to acquire a few samples from fisherman for use in their analyses; however, this method is not commonly used.

New trap design

Our trap design is derived from hellbender (*Cryptobranchus alleganiensis*) traps created by Briggler et al. (2013), which they modified from traps designed by Foster et al. (2008). Briggler et al. (2013) observed a few capture events of mudpuppies during tests of their traps; here we focused our efforts on the use of traps modified specifically for *N. maculosus*. The "Briggler traps" were constructed of aluminum wire and plastic mesh, with six panels bound together with zip ties to form a box. These traps were collapsible, with only 3-4 zip ties binding each panel together. Our traps are constructed from (9 Ga) aluminum wire, plastic net mesh, and zip ties. See Figure 1 for a list of materials per trap. Our traps have dimensions of 61 cm long x 46 cm wide x 22 cm tall, with a funnel diameter of 10 cm (Figure 1.2). Key modifications were made to improve ease of use, durability, and trap success. One modification was winding zip ties around the edges of the panels to bind them together. While this eliminated the collapsibility of the traps, it increased the durability. Because traps were no longer collapsible, we further modified the trap and added trap doors on the top of the trap to allow for the addition of bait and weight, as well as for the extraction of animals. Given that mudpuppies tend to keep their
limbs to the substrate, we used a thicker, more durable plastic mesh, with 1 cm holes, which potentially allows for a sturdier surface for a sturdier footing.

Our modified Briggler traps sat flush on the benthic substrate, enabling *N. maculosus* to walk up into the trap, rather than swim, potentially increasing the chance of capture relative to modified minnow traps. Time needed for construction of these traps was approximately 5-8 person hours per trap, though this process can be accelerated by forming a multi-person assembly line. Materials for these traps came to approximately $15 per trap, and materials can be purchased at most hardware stores.

To deploy, each trap was baited with raw fish scraps contained in a mesh bag (we used zip-tied plastic sleeves designed to pad wine bottles). Each trap was weighted by placing rocks found on the bank inside the trap, the trap door was zip-tied closed, and then placed on a flat part of the stream bed, preferentially in deep pools or next to large flat rocks. Traps were secured to the bank using 6 mm polypropylene rope tied to a tree or other stable structure. Each trap was left in the river for 1-2 nights. Manual surveys were also conducted, in which 2-4 surveyors walked/snorkeled upstream in rivers, lifting large flat rocks with and other potential refugia, and then capturing observed individuals by hand or with addition of a mesh bag.

Trapping was conducted for 528 trap-nights by deploying nine to ten traps at a time on a semi-regular basis from February 2014 to February 2015 (except for the months of April, May and August). We captured a total of 24 *N. maculosus* (Table 1.2), with a trap success of 0.045 *N. maculosus* per trap night. No *N. maculosus* were caught from June to September. All *N. maculosus* were caught between October and February 2015. Eliminating summer trapping hours results in 441 trap nights and a success rate of 0.054.
This success rate was comparable to some studies using modified minnow traps (McDaniel et al. 2009), and better than other trapping methods described above (Chellman and Parrish 2010; Matson 1990; Trauth et al. 2007; Palis 2010). Deploying and removing 10 traps required two people and approximately 2 hours per visit. Converting trap nights to person-hours equates to approximately 8 person-hours per trapping event, 4 person-hours for deployment, and 4 person-hours for collection. Our modified Briggler trapping took place over 232 person-hours and resulted in capture at a rate of 0.10 *N. maculosus* per person-hour (Table 1.2). Our modified Briggler trap method was more efficient than our manual surveys, which resulted in 49 *N. maculosus* over 1225 person-hours from May-September 2014 and October 2015, for 0.040 *N. maculosus* per person-hour. However, excluding a single highly productive site, at which we caught 33 *N. maculosus*, our manual survey success rate dropped to 16 *N. maculosus* over 924 person-hours, resulting in a capture rate of only 0.017 *N. maculosus* per person-hour.

**Conclusions**

Overall, sampling *N. maculosus* using any trapping method results in low capture rates, however trapping seems to work best from late fall through early spring (Bonin et al. 1995; Cagle 1954; Gendron et al. 1997; Matson 1990; Nickerson et al. 2002; Vandevalk and Coleman 2010). Late summer and fall seems to be an ideal time for manual surveys, as *N. maculosus* are relatively easily accessed due to larval guarding by females and the occurrence of breeding pairs under flat rocks and other cover objects, as well as generally low water levels (Hime et al. 2014; Petranka 1998). Winter through
mid-spring is a primary foraging period for \textit{N. maculosus} (Shoop and Gunning 1967), potentially explaining the higher trapping success rate during this time (McDaniel et al. 2009). Regardless of sampling method, researchers and managers need to be aware of the varying success rates based on time of year, and schedule their sampling dates accordingly.

\textit{Necturus maculosus} can occupy a wide range of habitats, from small streams to large rivers, and from small ponds to the Great Lakes (Bishop 1926; Matson 2005; Petranka 1998). This calls for flexibility in sampling methods depending on habitat type; manual surveys are most successful in clear and shallow water, seining works best in more debris-laden stream systems that are absent of large flat rocks, electroshocking works well in areas with few rocks and high conductivity, and trapping is ideal in deep and murky water, especially during the winter and early spring.

In conclusion, there is not a single, universally successful method for capturing \textit{N. maculosus} at all times of the year or in all habitats. It is vital that researchers and managers be flexible with \textit{N. maculosus} capture methods, and be prepared to utilize different methods for different habitat types and seasons. While not to be used as a single, paramount method, we suggest the addition of modified Briggler traps to the \textit{N. maculosus} capture arsenal, based on cost, time, and capture efficiency. Optimizing capture methodology will lead to the best chance for high capture rates, and will enable the further study of these understudied creatures.
Table 1.1. Summary of previous mudpuppy (*Necturus maculosus*) capture events. A “0” indicates that the method was tried, but with no capture success. A “-” indicates a method was used, but was largely ineffective and/or not recommended. A “+” indicates that a method was used and was successful and/or recommended. **caught using fishing poles rather than traditional set lines.**

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Location</th>
<th>Time of Year</th>
<th>Electroshocking</th>
<th>Manual Survey</th>
<th>Minnow Trap</th>
<th>Seine</th>
<th>Trapnet</th>
<th>Set Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cagle</td>
<td>1954</td>
<td>Big Creek, LA</td>
<td>Jan-Feb</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoop and Gunning</td>
<td>1967</td>
<td>Big Creek, LA</td>
<td>Year-round</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gibbons and Nelson Jr</td>
<td>1968</td>
<td>Gull Lake, MI</td>
<td>Apr-May</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Matson</td>
<td>1990</td>
<td>Grand River, OH</td>
<td>Mar-July</td>
<td>0</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bonin et al.</td>
<td>1995</td>
<td>St. Lawrence River, Can.</td>
<td>Winter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+**</td>
</tr>
<tr>
<td>Nickerson et al.</td>
<td>2002</td>
<td>Little Pigeon River, TN</td>
<td>Aug-Oct</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Schmidt et al.</td>
<td>2004</td>
<td>Hudson River, NY</td>
<td>Summer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Harper et al.</td>
<td>2006</td>
<td>West-Central MN</td>
<td>May, Jun, Sep</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Trauth et al.</td>
<td>2007</td>
<td>Spring River, AR</td>
<td>Year-round</td>
<td>+</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McDaniel et al.</td>
<td>2009</td>
<td>Sydenham River, ON</td>
<td>Nov-Mar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Chellman and Parrish</td>
<td>2010</td>
<td>Lamoille River, VT</td>
<td>Year-round</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>VanDe Valk and Coleman</td>
<td>2010</td>
<td>Northern NY</td>
<td>Oct-Nov (Apr)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Palis</td>
<td>2010</td>
<td>Lusk Creek, IL</td>
<td>Sep-Oct, May-Jun</td>
<td>-</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1.2. Summary of our mudpuppy (*Necturus maculosus*) sampling for both manual surveys and trapping surveys using modified Briggler traps. Month is indicated by first letter. Absence of a number indicates no sampling took place in that watershed during that month. No sampling took place during March or April.

<table>
<thead>
<tr>
<th>Basin</th>
<th>Method</th>
<th>Total Person-Hours</th>
<th>Total caught</th>
<th>J</th>
<th>F</th>
<th>-</th>
<th>M</th>
<th>J</th>
<th>J</th>
<th>A</th>
<th>S</th>
<th>O</th>
<th>N</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manual</td>
<td>353</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kentucky</td>
<td>Trapping</td>
<td>120</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Manual</td>
<td>41</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinni-</td>
<td>Trapping</td>
<td>8</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>conick</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Licking</td>
<td>Manual</td>
<td>621</td>
<td>36</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trapping</td>
<td>104</td>
<td>16</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1.1. Materials needed per trap for the construction of modified Briggler traps for the common mudpuppy (*Necturus maculosus*).

- 9 gauge Galvanized Steel Wire (528"/trap, 44')
  - 2 x 90"
  - 2 x 70"
  - 2 x 68"
  - 2 x 36"

- Plastic Fence (1 cm square holes)
  - 1 x (24" x 56")
  - 2 x (14" x 18")

- Plastic Zip Ties
  - 48 x 8"
  - 75 x 22"
Figure 1.2. Modified Briggler trap. Note the trap door on top for accessing trap compartment, as well as funneled ends which allow for mudpuppies to walk into the trap while positioned on stream floor.
Figure 1.3. Mudpuppy (*N. maculosus*) captured in trap near Cynthiana, Kentucky USA.
CHAPTER TWO

POPULATION STRUCTURE IN A PERMANENTLY AQUATIC SALAMANDER ACROSS MULTIPLE SPATIAL SCALES: THE ROLE OF BARRIERS AND RIVER ARCHITECTURE

Introduction

Population structure varies across a spectrum of divergence, with panmictic populations on one end of the spectrum, and completely isolated populations on the other (Wright 1949; Hutchinson 1999). This structure is a balance between gene flow and isolation, and the evolution of population structure across a species’ range can be influenced by a complex interaction of both intrinsic factors, such as a species’ life history, and extrinsic factors, such as landscape architecture and anthropogenic barriers (Coulon et al. 2013; Finn et al. 2007). Extrinsic factors in particular have the potential to affect patterns of population structure differentially across a species’ range, with local landscapes offering different levels of resistance to the movement of individuals (Zeller 2012). This heterogeneity across local geographic scales could lead to an assortment of patterns of population structure across a species’ range (Husemann 2012); however, studies of most species are typically characterized by a single model to explain their overall patterns of population structure. Here, I examine the role of spatial scale and extrinsic factors responsible for population structure in an aquatic salamander.

Recently, studies have begun to investigate how population structure develops in lotic (river and stream) systems, though much is still unknown about how genetic variation is partitioned across these landscapes. Lotic systems can differ substantially
from terrestrial landscapes, with gene flow among populations primarily restricted to those connected through a hierarchical aquatic network, with small, lower order streams combining to form larger, higher order streams. As such, lotic networks offer limited and known dispersal paths between populations and can impose structure at multiple spatial scales. Smaller streams join to form larger streams, creating a similar branching network architecture at multiple spatial scales. This network architecture is known to constrain ecological, demographic, and evolutionary processes (Lowe et al. 2006; Campbell Grant et al. 2007). Hughes (2007) found that animals wholly restricted to the stream channel generally exhibited very strong population structure between basins and was especially pronounced in species limited to lower order streams. Alternatively, species that disperse out-of-network, such as salamanders with a terrestrial dispersal life-history stage or insects with a flight stage, typically exhibited lower population structure (Hughes et al. 2009; Campbell Grant et al. 2007; Steele et al. 2009).

While many of these studies have found specific patterns of population structure, they have been generally limited in spatial scale, and have not examined the effects of local barriers across a broader distributional range. Even so, a number of generally accepted models of population structure have been identified for lotic species. Two models in particular best capture the range of population structure evolution: (1) population structure is hierarchically organized with nested patterns of increasing structure with increasing levels of stream hierarchy from streams, to catchments, to basins, influenced by a positive relationship between gene flow and network proximity (e.g., the stream Hierarchy Model (Meffe and Vrijenhoek 1988). This type of structuring has been broadly found in a range of organisms that are restricted to the stream channel,
including invertebrates, fish, and salamanders (Bilton et al. 2001; Castric et al. 2001; Steele et al. 2009). Alternatively, populations are isolated along all orders of stream networks with very little gene flow regardless of network hierarchy, resulting in population structure driven largely by genetic drift e.g., the Death Valley Model (Finn et al. 2007; Mullen et al. 2010).

While a single model may be useful in understanding patterns of genetic structure among local populations, larger scale patterns of population structure across a species’ range may not be best described by a single model, but rather a combination of multiple models. A species’ population structure may largely be described by one model, but local dispersal barriers may elicit differential patterns of population structure in part of a species’ range. For example, the construction of dams and other hydrological impoundments can act as dispersal barriers and hinder gene flow among populations, dramatically changing population structure, much like in the Death Valley Model (Tiemann et al. 2004; Yamamoto et al. 2004; Allan & Castillo, 2007). In the same manner, Pleistocene glaciation has strongly driven historical population isolation (Petit et al. 2003; Costello et al. 2003), creating patterns consistent with the Death Valley Model within a broader hierarchical structure. In another example, the population structure of facultatively paedomorphic species can have elements of both the Stream Hierarchy Model, which accounts for aquatic dispersal, and the Headwater Model, a separate model that accounts for overland movement between nearby headwater streams (Steele et al. 2009). The spatial arrangement of lotic systems, individual species’ dispersal modes and life history strategies, as well as local barriers can all shape patterns of population structure across a species’ range (Lowe et al. 2006; Hughes et al 2009). In this study, I
provide a new perspective on the heterogeneous evolution of population structure in a lotic system by examining genome-wide patterns of population structure in a fully aquatic salamander across multiple basins, offering a first look at potential effects of network hierarchy and dams on the population structure of an aquatic salamander. 

*Necturus maculosus* is an obligate paedomorphic salamander, native to eastern North America (Bartlett and Bartlett 2006). While geographically widespread and presumably common (Barbour 1971; Petranka 1998), the population status of *N. maculosus* is poorly understood over most its range. There is a current lack of basic information on this species, including habitat preferences, seasonal movements (Pope 1947; Gibbons and Nelson Jr. 1968; Green and Pauley 1987), population structure, gene flow and dispersal (but see McDaniel et al. 2009; Chellman and Parrish 2010). While many salamander species are able to disperse terrestrially between streams, with dispersal significantly impacting patterns of gene flow (Miller et al. 2015), *N. maculosus* are wholly restricted to their aquatic environment, and do not disperse overland (Petranka 1998). *Necturus maculosus* can, however, inhabit a wide variety of habitats, from small streams to large rivers, and from ponds to the Great Lakes. This flexibility in use of habitat could affect overall patterns of population structure, and potentially allow for this species to be minimally influenced by barriers for dispersal.

The recovery of sufficient levels of genetic variation is a key factor in accurately estimating fine-scale population genetic processes. Traditional molecular markers used in population genetic studies [e.g., mitochondrial DNA (mtDNA) sequence data, microsatellites, RFLPs, AFLPs or allozymes], have been limited in their number of independent markers they can provide for detecting patterns of population structure that
have evolved over recent time scales (Catchen et al. 2013). MtDNA, in particular, has been one of the most commonly used markers for studying population genetic variation in lotic systems, yet it represents only a single locus, and does not provide insight into the independent evolution of the nuclear genome (Moore 1995). Microsatellites have served as an accessible multilocus approach, and generally offer high levels of allelic variation and heterozygosity to differentiate populations (Shaw et al. 1999), but are expensive and time consuming to produce, and are still limited as a genome-wide assessment of variation (Vignal et al. 2002; Catchen et al. 2013). High throughput next generation sequencing (NGS) allows for the collection of thousands of independently evolving single nucleotide polymorphisms (SNPs), without the need to develop new markers for each species (Hohenlohe et al. 2011), and without the need for prior genome sequence information (Hohenlohe et al. 2012). Moreover, NGS methods have the potential to generate orders of magnitude greater data than previous genetic methods, providing the arsenal of genomic variation needed to tease apart the population processes acting to structure genetic variation at fine geographic scales (McCormack et al. 2013). This large sample of genomic variation also enables inferences of population structure in populations that exhibit heterogeneity across genomes due to events such as recent demographic shifts, as well the detection of weak population structure caused by recent changes in gene flow and genetic drift (Anderson et al. 2010; Catchen et al. 2013). In order to understand, monitor, and restore lotic-adapted species, the use of powerful genomic data sets is crucial in understanding the interaction between the dispersal traits of species, the structure and influence of dendritic riverine networks, and the effects of anthropogenic barriers.
Here, I aimed to explore the population structure of a widespread, aquatic salamander, *N. maculosus*, to understand the role that spatial scale has on interpreting models of population structure, and the relative influence of network architecture and dispersal barriers on determining best-fit models of population structure at multiple scales. I provided a first assessment of population structure in this widespread salamander across three basins, and looked for patterns of population structure that may fit one or more models. Specifically, I examined the influence of stream network architecture and dispersal barriers, looking for patterns of population structure that best fit the Stream Hierarchy Model or the Death Valley Model by examining how genetic structure was partitioned across all levels of sampling, including the highly impounded Kentucky River basin, and the less impounded Kinniconick and Licking River basins.

**Methods**

*Sampling sites and design*

To examine the spatial extent of population structure in *N. maculosus*, samples were collected at two hierarchical scales: basins and catchments. Specifically, *N. maculosus* were sampled within three major river basins in eastern and central Kentucky: the Licking River, Kentucky River, and the unbranched Kinniconick Creek located in northeastern Kentucky. Each of these three basins flow directly into the Ohio River (Figure 2.1). Within each basin, I collected tissue samples of *N. maculosus* at 1 to 5 sites, where each site generally represented a different catchment (Table 2.1). The study sites varied in terms of distance between the next closest site, ranging from 6 to 1022 km.
measured using the National Inventory of Dams measuring tool (USACE 2013) (Table 2.2). Two sites in the Red River (a stream in the Kentucky River basin) were within 1 km of each other and were treated as one site for this study. To look for any effects due to dams and impoundments as potential barriers for dispersal, basins were chosen with a wide range of damming, from the heavily impounded Kentucky River, to the much less disturbed Licking River and Kinniconick Creek. Sites were separated by zero to thirteen dams (USACE 2013) (Table 2.3; Figure 2.1).

At each site, *N. maculosus* were captured using manual snorkel surveys and trapping, depending on the season. At each site 1-7 tissue samples were collected via tail clipping from both adult and larval *N. maculosus* and stored in 95% ethanol. A total of 41 individuals were collected from 10 sites. All tissue sampling took place between August 2013 and September 2015. For a full description of field methods, see Murphy et al. (in press; Chapter 1).

*Genetic data collection*

A limiting factor in the generation of population genomic data from salamanders using an NGS method has been their large genome size (Gregory 2001). This is particularly true for *N. maculosus*, with a genome size estimated at 85 gigabases. I overcame this issue using double-digest restriction site-associated DNA (ddRAD) sequencing (Peterson et al. 2012), which is a reduced-representation NGS method that focuses sequencing effort on a subset of the genome that is flanked by restriction enzyme cutting sites. This permits the recovery of tens of thousands of orthologous loci across sampled individuals within a species, with substantial recovery of SNPs. Given the large
genome size of *N. maculosus*, a larger amount of starting DNA (3000 ng) was used for library preparation compared to the standard protocol amount (100-1000 ng) suggested in Peterson et al. (2012). Genomic DNA was digested from 41 individuals using two different restriction enzymes, SphI and EcoRI. Digested fragments were barcoded through the ligation of individual-specific index sequences and then size selected for a mean fragment size of 376 bp using a PippenPrep machine (Sage Science). Collectively, these steps result in the generation of a library containing a reduced set of fragments from each individual, increasing the probability that recovered loci will have high sequence coverage on an Illumina HiSeq platform, and increasing the probability that similar sets of orthologous loci are sequenced across multiple individuals. Pooled libraries were paired-end sequenced (150 bp) on an Illumina 2100 HiSeq. Even with this ddRAD approach, the large genome size of *N. maculosus* still limits the number of individuals that can be multiplex sequenced on a single HiSeq lane. As a result, 10-11 individuals were multiplex sequenced per lane, which permitted the recovery of substantial overlap in sequence reads across all individuals and all loci.

Paired-end sequence reads (R1 and R2) were initially analyzed using Stacks v.1.35 (Catchen et al. 2011) to identify the total set of loci within individuals and shared orthologous loci across individuals. Reads from each individual were first concatenated into two files by forward (R1) and reverse (R2) read designation, were stitched together using a custom script (Appendix), and then filtered for quality using `process_radtags` in Stacks. Reads were removed if they contained uncalled bases, or if they contained a mean quality score < 20 within a sliding window of 15% of the read length. Reads passing quality filtering were then *de novo* assembled with a minimum stack (i.e. number of
reads) depth of four and a maximum of four mismatches permitted between loci. Further filtering was conducted in the Stacks-based populations program, by reducing loci to those found in every individual and with a minimum stack depth of five reads. For loci with multiple SNPs, the Stacks flag --write_random_snp was used to ensure only one SNP was selected from each locus. As a final step, the program VCFtools v0.1.14 (Danecek et al. 2011) was used to remove all loci with a minimum mean depth < 10 and a maximum mean depth > 250.

*Estimating genetic diversity*

Genomic diversity was assessed at each of the 10 study sites by calculating observed heterozygosity (H_o), expected heterozygosity (H_e), and nucleotide diversity (\(\Pi\)). The relative number of heterozygotes at each site was calculated using Wright’s inbreeding coefficient (F_{IS}). To assess for signatures of demographic expansion or contraction, Tajima’s D was calculated with all sites combined, as well as at the site and basin level using the PopGenome package in R v.3.2.3 (Pfeifer et al. 2014), using the 95% confidence interval around 0 for a rough estimate of significance i.e.(-2 > D > 2; Anholt and Mackay 2009). Divergence between sites was assessed using pairwise F_{ST} statistics. All summary statistics, with the exception of Tajima’s D, were calculated in Stacks v.1.35 (Catchen et al. 2013).

*Assessment of population structure*

Population structure was explored with two programs, one using a model based approach (ADMIXTURE; Alexander et al. 2009), and one using a semi-model based
approach that first incorporates a principal components analysis (DAPC; Jombart 2010). Both of these methods allow for the exploration of population structure independently of pre-defined basin assignment, and allow for an assessment of individuals with admixed genomic variation from two or more populations. First, ADMIXTURE was used to assign individuals to populations using a maximum likelihood approach based on a block relaxation approach and a sequential quadratic programming algorithm. Appropriate numbers of populations was checked using cross-validation error, with low error indicating a higher probability of that number of population being accurate. Results were visualized with the program Clumpak (Kopelman et al. 2015). Second, a discriminant analysis of principal components (DAPC) was performed using the adegenet package in R to assess the relative degree to which populations are genetically structured across the study area. This is a semi-model based approach that uses principal components analysis to describe genetic clusters using synthetic variables called discriminant functions. Each site was treated independently and sites were grouped together in populations selected by the find.clusters program. The number of principal components and discriminant functions was determined by performing 1000 replicates of cross-validation using the R package poppr (Kamvar et al. 2014). Results from replicate analyses were used to determine the highest mean assignment success and lowest mean standard error.

To examine how genetic variation was partitioned at different hierarchical scales, Analyses of MOlecular VAriance (AMOVA) were performed in the R package pegas (Meirmans 2006; Paradis et al. 2015). Two different sets of analyses were performed: (1) an analysis using populations selected a priori by river basin, and (2) an analysis where populations were defined based on ADMIXTURE and DAPC results. In both sets of
AMOVA analyses, the degree to which genetic variation was partitioned across three levels was assessed: between basins (or clusters in the second analysis), between sites within basins/clusters, and within sites.

Mantel tests were used to calculate the correlation between genetic distance and geographic distance, permitting non-parametric tests of a model representing the role of Isolation By Distance (IBD) among populations. In addition, partial Mantel tests were used to test for a correlation between genetic distance and geographic distance, while accounting for the number of dams between sites and basin assignment, serving as a test of the role of these factors in driving population structure. All Mantel and partial Mantel tests were performed in the R package `vegan` package (Oksanen et al. 2007) using 9,999 matrix randomizations.

**Results**

A total of 1,439,623 loci were recovered using the STACKS pipeline. Quality filtering resulted in 9,694 unlinked SNPs shared across all individuals at a minimum stack depth of 5x, and a mean coverage depth of 50.6 reads per individual. \( H_o \) varied among populations from 0.097-0.147, and \( H_e \) ranged from 0.055-0.126. Nucleotide diversity (\( \Pi \)) was similar to \( H_o \), ranging from 0.106 to 0.145. \( F_{IS} \) values were generally negative, but not significantly different from zero (Table 2.4). Pairwise \( F_{ST} \) between sampling sites ranged from 0.061 to 0.264 and were generally greater between basins (Table 2.5). When calculated across all populations, Tajima’s \( D \) was -0.78, indicating that there was less variation than expected, and that populations might be expanding after a bottleneck. Tajima’s \( D \) at the basin and site level were also generally negative, though a
few sites had positive values (Table 2.6.) Overall, no value of Tajima’s D was substantially large or small (i.e., < -2 or > 2).

ADMixTURE results supported the clustering of localities into 2-5 populations based on cross-validation scores. The strongest support was for two populations (K = 2), but clustering levels up to a K =5 also received support (Figure 2.2). Furthermore, these additional levels of population clustering broke out along basins and catchments. At a K = 2 level, populations split strongly between the Kentucky River basin and the combined Kinniconick Creek and Licking River basin. Under a K = 3 model, the Sturgeon Creek site (locality 10) separated from the Kentucky River basin and the combined Kinniconick Creek and Licking River basin. At K = 4, the Kinniconick population was identified as a distinct population, and at K = 5, the combined main stem Licking site and the South Fork Licking site (localities 2 and 3), which are only 15.5 km apart, were identified as distinct from other Licking River basin sites (Figure 2.3). In the discriminant analysis of principal components, cross-validation found the best number of principal components to be 20, leaving 1 linear discriminant, which together captured 71.9% of the conserved variance. Similar to the ADMIXTURE results, the Kentucky River sites clustered together, as did the Licking River and Kinniconick Creek populations (Figure 2.4).

Results from AMOVA using the three river basins as the highest level of hierarchical structure resulted in a significant amount of genetic variation being attributed to the among-site (11.79%) and among-individual (85.24%) levels, but not between basins (Table 2.7). A two-population AMOVA based on the best-supported level of population structure in ADMIXTURE and DAPC analyses indicated significant structure
at all levels (Table 2.7) with the majority of genetic variation accounted for at the among-individual level (83.1%).

Simple Mantel tests showed a positive correlation between genetic distance and geographic distance ($p < 0.001$, $r = 0.607$), and a positive correlation between genetic distance and number of dams between sites ($p < 0.001$, $r = 0.715$). Partial Mantel tests revealed that while there was no significant correlation between genetic distance and geographic distance when controlling for the effect of dams between sites ($p = 0.692$, $r = -0.105$), there was a significant correlation between genetic distance and dams when controlling for geographic distance ($p < 0.019$, $r = 0.484$). Finally, partial Mantel tests found a correlation between geographic and genetic distance, and between genetic distance and the number of dams, when controlling for the effect of river basin ($p = 0.044$, $r = 0.404$; $p = 0.009$, $r = 0.700$). Tests within basins found a positive correlation between genetic and geographic distance among the combined Licking/Kinniconick sites, controlling for the number of dams ($p < 0.044$, $r = .483$). Within the Kentucky River basin, no significant relationship was detected between genetic distance and either geographic distance or the number of dams ($p = 0.17$, $r = 0.453$; $p = 0.17$, $r = 0.490$).

**Discussion**

I found evidence for a multi-model explanation of population structure for *N. maculosus* influenced by network architecture and spatial scale, as well as dispersal barriers, which corresponds to the Stream Hierarchy Model and Death Valley Model, respectively.
Stream Hierarchy Model

*Necturus maculosus* generally exhibited hierarchical population structure, with significant structuring at the cluster and site levels ($p < 0.018$). Population structure showed strong divergence between the Kentucky River basins and the combined Licking/Kinniconick cluster (Figure 2.4, Table 2.5), though further partitioning of structure occurred within clusters and by basin, which suggest that a $K = 5$ level may best describe the system (Figure 2.2). Based on previous studies of freshwater fish (Castri et al. 2001; Hughes 2007), we would expect to find relatively high measures of $F_{ST}$ in fully aquatic organisms. *Necturus maculosus* had high $F_{ST}$ values among basins relative to within basins and a mean $F_{ST}$ of 0.14 between all sites. The patterns and scope of $F_{ST}$ values in *N. maculosus* are consistent with other fully aquatic stream salamanders: *Cryptobranchus alleganiensis bishopi* (mean $F_{ST} = 0.40$), *Dicamptodon copei* (mean $F_{ST} = 0.079$), and *Cryptobranchus alleganiensis alleganiensis* (mean $F_{ST}$ 0.067) (Crowhurst et al. 2011; Steele et al. 2009, Unger et al. 2013). High divergence between stream basins was a consistent pattern in all fully aquatic stream salamanders, and sites in different basins typically had the highest values.

The population structure of *N. maculosus* largely corresponds to the Stream Hierarchy Model, with genetic variation partitioned among sites, with greater variation among basins. In the Stream Hierarchy Model, network architecture greatly impacts population structure. Meffe and Vrijenhoek (1988) demonstrated that for species restricted to a continuously connected dendritic network, population structure is influenced by geographic proximity, with the stronger population structure among basins.
than within basins. Similar patterns of structure have been found in other stream salamanders. Steele et al. (2009) found evidence for hierarchical structuring influenced by geographic proximity in a fully aquatic salamander, with evidence for eight population clusters organized by basin. The population structure of *C.a.alleganiensis* mirrors that of *N. maculosa*, with significant structure at multiple spatial scales, the presence of two strongly supported population clusters organized by basin, and the presence of IBD within basins (Unger et al. 2013). Like other species which exhibit hierarchical population structure, *N. maculosa* is wholly restricted to the water column, and does not exhibit overland dispersal. Though *N. maculosa* can inhabit a variety of habitats (Petranka 1998), and is suggested to exhibit seasonal migration (Matson 1998), adults often exhibit high site fidelity (Matson 1998; Shoop and Gunning 1967), and individuals are still subject to the network architecture of the lotic system, which constrains dispersal between catchments and basins accounting for geographic proximity. This is in contrast to species exhibiting out-of-network dispersal, which are able to supersede network architecture, and exhibit lower levels of population structure, such as *D.tenebrosus* (mean F<sub>ST</sub> = 0.031, and no evidence of population structuring (Steele et al. 2009), as well as species that exist in spatially proximate, yet isolated populations, due to limited dispersal capabilities, habitat specialization, or the presence of dispersal barriers (Meffe and Vrijenhoeck 1988; Finn et al. 2007; Hughes et al. 2009).

**Death Valley Model**

I also found evidence for further structuring that is not a result of network architecture. Though the distance between the Kinniconick Creek site and the Licking
River sites was comparable to the distance between the Kentucky and Licking River sites (Table 2.2), the Kinniconick site was consistently grouped with the Licking sites, which was not consistent with the Stream Hierarchy Model. This may however be a remnant of a split between ancient rivers, where the Old Kentucky basin was separated from the ancient Teays River, which flowed north and contained what are now the Licking and Kinniconick basins (Teller 1973; Teller and Goldthwait 1991).

The structure between the Sturgeon Creek population and the rest of the Kentucky River basin however provides evidence for population structure influenced by factors other than network architecture. The Sturgeon population diverged from other sites in the Kentucky drainage, even though it is located relatively in the center of the range (Figure 2.1). This could be explained by local barriers isolating this population, which follows the Death Valley Model of population structure. I speculate that the large number of dams in the Kentucky River basin relative to the combined Licking/Kinniconick sites resulted in this structure. Dams significantly affect the dispersal and population structure of aquatic species (Fullerton et al. 2010; Nislow et al. 2011). Neraas et al. (2001) found damming severely restricted movement and isolated bull trout (*Salvelinus confluentus*) populations. Bessert and Ortí (2008) examined the population structure of the blue sucker, *Cycleptus elongatus*, in two large watersheds: the Missouri River, which is significantly impounded allowing for only unidirectional movement, and the Mississippi River which does not have impassable dams. Though the Missouri and Mississippi Rivers have similar hydrological qualities, they found significant structure in populations within the Missouri River not present along a similar stretch of habitat on the Mississippi River. Though an effect of damming has been widely reported in fish species, my study suggests that the
damming of the Kentucky River could affect *N. maculosus* in a similar manner, with genetic structure best described by the Death Valley Model in parts of the Kentucky River basin.

Population structure corresponding to the Death Valley Model typically occurs in species restricted to headwaters with limited dispersal ability (Meffe and Vrijenhoek 1988; Finn et al. 2006; Finn et al. 2007; Hughes et al. 2009), though Mullen et al. (2010) identified similar high structure consistent with the Death Valley Model between catchments. This study highlights the possibility that structure similar to the Death Valley Model can be driven by barrier induced dispersal restrictions, resulting in a larger influence of genetic drift on population structure.

*Influence of scale on multiple models*

Scale is important to consider when examining patterns of population structure and gene flow of any species (Anderson et al. 2010). When considering the scale for analyses, we must not only consider the landscape architecture and its effects over broader distances, but also barriers for dispersal, both ancient and recent (Zellmer and Knowles 2009). It is important to recognize that at a local scale, a single model may best describe population structure, but when as scale increases, different barriers in the landscape may act on a species, necessitating a multi-model approach.

Previous studies have shown support for multiple explanatory models depending on scale. Mullen et al. (2010) examined population structure of a facultative paedomorphic salamander, and found that among-site structure was largely driven by genetic drift (in support of the Death Valley Model), but that within-site structure was
driven by gene flow among streams (in support of the Stream Hierarchy Model). Dudaniec et al. (2012) found inherent landscape features acted to uniquely alter the dispersal pathways of *D.tenebrosus* over separate parts of its geographic range.

Previous studies of fully aquatic salamanders have found hierarchical patterns of population structure consistent with the Stream Hierarchy Model (Steele et al. 2009; Unger et al. 2013). I found similar patterns in areas with relatively few dispersal barriers, such as in the Licking/Kinniconick cluster. In this cluster, *N. maculosus* exhibited significant relationship between genetic and geographic distance (*p* < 0.001). In contrast, there was no such relationship in the heavily impounded Kentucky River basin. I show that dispersal barriers can have a significant effect on population structure by limiting and altering species specific dispersal patterns, thus indicating a multi-model explanation of population structure. Within my study area, I found evidence for two models of population structure. Damming on the Kentucky river has imposed genetic structure consistent with the Death Valley Model, whereas relatively little damming within the Licking/Kinniconick watersheds has led to less isolated populations, IBD, and population structure that generally conforms to the Stream Hierarchy Model.

In my study, *N. maculosus* generally followed the Stream Hierarchy Model for population structure, though this model did not capture the entire pattern of population structure in the system. Local barriers may have caused some populations to fit the Death Valley Model, where differentiation developed without correlation to drainage pattern, such as in Sturgeon Creek. Furthermore I presented evidence that the impounding of the Kentucky River has led to increased isolation from other basins. Overall, *N. maculosus* in eastern Kentucky seem to be partitioned into multiple general models of population
structure. When examined at a broader scale, patterns of gene flow and structure may be
best represented by a patchwork of local processes, rather than a single overarching
model.

Other demographic factors

Similar to *D. aterrimus* (Mullen et al. 2005), *N. maculosus* may have relatively
small population sizes within my sampling area, as sampling effort was considerable to
obtain just 1-7 individuals per site (Murphy et al. in press; Chapter 1). In addition, overall
values of $H_o$ were relatively low at each site (mean = 0.121) relative to values found in *D.
aterrimus* (mean = 0.36, Mullen et al. 2010) Though high $H_o$ was found in *C. a.
allemaniensis* (mean = 0.82), Unger et al. (2013) suggests this is due to the confounding
factors of historically larger populations and the unusually long lifespan of *C.
allemaniensis*. Sampling effort and low $H_o$ could indicate small populations. Nevertheless,
across my study area, *N. maculosus* may be experiencing population growth, potentially
due to expansion or reconnection following a previous bottleneck or isolation event
which reduced overall genomic variation, supported by both higher $H_o$ than $H_e$ and
weakly negative estimates of Tajima’s D (Tables 4, 6). Even so, the Sturgeon site had a
weakly positive Tajima’s D, which supports recent population decline, and may
indicating drift may play a larger role in the genomic variation in this population. Overall,
measures of population size and demography in *N. maculosus* are unclear and warrant
further study.
Conclusions

I offer a first look at the population structure of *N. maculosus*, and examine potential effects of extrinsic factors on patterns of population structure among and within three river basins. By examining population genomic variation in *N. maculosus*, my study furthers understanding of the evolutionary implications of structure within a hierarchical dendritic network, and further explores the interaction and effect study scale, network architecture, and local dispersal barriers have on population structure. Furthermore, it provides the framework for accurately estimating the population processes that are critical to management of *N. maculosus* and could lead to the identification of distinct population units. Though some studies suggest treating models separately, and managing populations accordingly (Meffe and Vrijenhoek 1988), I suggest an integration of multiple models to best understand patterns of population structure over large scales, which may be especially useful for imperiled species. In order to understand, monitor, and restore riverine landscapes and species, we must better understand the interaction between species’ dispersal traits, the structure and limitations of these dendritic networks, any effects wrought by local historical or anthropogenic barriers, and the scale by which all of these factors are examined.
Table 2.1: Sites sampled with corresponding site abbreviation, site number, basin location, individuals captured, and GPS coordinates.

<table>
<thead>
<tr>
<th>Site name</th>
<th>Site abbr.</th>
<th>Site number</th>
<th>Basin</th>
<th>Number captured</th>
<th>Lat</th>
<th>Long</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinniconick Creek</td>
<td>Konnick</td>
<td>1</td>
<td>Kinniconick</td>
<td>5</td>
<td>38.54439</td>
<td>-83.2255</td>
</tr>
<tr>
<td>Licking River</td>
<td>LFal</td>
<td>2</td>
<td>Licking</td>
<td>6</td>
<td>38.67752</td>
<td>-84.2983</td>
</tr>
<tr>
<td>South Fork Licking River</td>
<td>SFL</td>
<td>3</td>
<td>Licking</td>
<td>4</td>
<td>38.64188</td>
<td>-84.3775</td>
</tr>
<tr>
<td>N. Fork Tripplett Creek 2</td>
<td>Trip2</td>
<td>4</td>
<td>Licking</td>
<td>5</td>
<td>38.26099</td>
<td>-83.4338</td>
</tr>
<tr>
<td>Craney Creek</td>
<td>Craney</td>
<td>5</td>
<td>Licking</td>
<td>4</td>
<td>38.07006</td>
<td>-83.3435</td>
</tr>
<tr>
<td>N. Fork Tripplett Creek 1</td>
<td>Trip1</td>
<td>6</td>
<td>Licking</td>
<td>6</td>
<td>38.24627</td>
<td>-83.4389</td>
</tr>
<tr>
<td>Gladie Creek</td>
<td>RRG</td>
<td>7</td>
<td>Kentucky</td>
<td>4</td>
<td>37.83688</td>
<td>-83.6085</td>
</tr>
<tr>
<td>Stanton Creek</td>
<td>Stanton</td>
<td>8</td>
<td>Kentucky</td>
<td>2</td>
<td>37.83709</td>
<td>-83.8871</td>
</tr>
<tr>
<td>Sturgeon Creek</td>
<td>Sturgeon</td>
<td>9</td>
<td>Kentucky</td>
<td>4</td>
<td>37.54183</td>
<td>-83.7805</td>
</tr>
<tr>
<td>Greasy Creek</td>
<td>Greasy</td>
<td>10</td>
<td>Kentucky</td>
<td>1</td>
<td>36.99268</td>
<td>-83.2957</td>
</tr>
</tbody>
</table>
Table 2.2. Geographic distance (km) matrix between sampling sites.

<table>
<thead>
<tr>
<th></th>
<th>LFal</th>
<th>SFL</th>
<th>Trip2</th>
<th>Craney</th>
<th>Trip1</th>
<th>RRG</th>
<th>Stanton</th>
<th>Greasy</th>
<th>Sturgeon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Konnick</td>
<td>268.1</td>
<td>277.6</td>
<td>484.0</td>
<td>517.5</td>
<td>490.0</td>
<td>702.5</td>
<td>667.3</td>
<td>866.0</td>
<td>709.9</td>
</tr>
<tr>
<td>LFal</td>
<td>15.5</td>
<td>215.9</td>
<td>249.4</td>
<td>221.9</td>
<td>609.2</td>
<td>574.0</td>
<td>772.7</td>
<td>616.6</td>
<td></td>
</tr>
<tr>
<td>SFL</td>
<td>231.4</td>
<td>6.0</td>
<td>237.4</td>
<td>618.7</td>
<td>583.5</td>
<td>782.2</td>
<td>626.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trip2</td>
<td>76.7</td>
<td>264.9</td>
<td>237.4</td>
<td>825.0</td>
<td>789.8</td>
<td>988.5</td>
<td>832.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Craney</td>
<td>82.7</td>
<td>858.6</td>
<td>823.4</td>
<td>1022.1</td>
<td>866.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trip1</td>
<td>831.0</td>
<td>795.8</td>
<td>994.5</td>
<td>838.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRG</td>
<td>35.2</td>
<td>344.7</td>
<td>188.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stanton</td>
<td>309.5</td>
<td>153.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greasy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>162.1</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.3. Total number of dams separating sampling sites.

<table>
<thead>
<tr>
<th></th>
<th>LFal</th>
<th>SFL</th>
<th>Trip2</th>
<th>Craney</th>
<th>Trip1</th>
<th>RRG</th>
<th>Stanton</th>
<th>Greasy</th>
<th>Sturgeon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Konnick</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>LFal</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>SFL</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>12</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trip2</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>12</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Craney</td>
<td>1</td>
<td>8</td>
<td>8</td>
<td>13</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trip1</td>
<td>7</td>
<td>7</td>
<td>12</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRG</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stanton</td>
<td>5</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greasy</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.4. Summary statistics for all ten sampling sites, including number of individuals (Num INDV), Observed Heterozygosity ($H_o$), Expected Heterozygosity ($H_e$), and nucleotide diversity ($\pi$).

<table>
<thead>
<tr>
<th>Pop ID</th>
<th>Num INDV</th>
<th>$H_o$</th>
<th>Var</th>
<th>$H_e$</th>
<th>Var</th>
<th>$\pi$</th>
<th>Var</th>
</tr>
</thead>
<tbody>
<tr>
<td>Konnick</td>
<td>5</td>
<td>0.120</td>
<td>0.045</td>
<td>0.100</td>
<td>0.026</td>
<td>0.111</td>
<td>0.033</td>
</tr>
<tr>
<td>LFal</td>
<td>6</td>
<td>0.137</td>
<td>0.039</td>
<td>0.121</td>
<td>0.026</td>
<td>0.132</td>
<td>0.030</td>
</tr>
<tr>
<td>SFL</td>
<td>4</td>
<td>0.119</td>
<td>0.048</td>
<td>0.098</td>
<td>0.027</td>
<td>0.112</td>
<td>0.035</td>
</tr>
<tr>
<td>Trip2</td>
<td>5</td>
<td>0.116</td>
<td>0.041</td>
<td>0.102</td>
<td>0.026</td>
<td>0.114</td>
<td>0.033</td>
</tr>
<tr>
<td>Craney</td>
<td>3</td>
<td>0.097</td>
<td>0.040</td>
<td>0.091</td>
<td>0.027</td>
<td>0.109</td>
<td>0.038</td>
</tr>
<tr>
<td>RRG</td>
<td>4</td>
<td>0.147</td>
<td>0.049</td>
<td>0.126</td>
<td>0.029</td>
<td>0.144</td>
<td>0.038</td>
</tr>
<tr>
<td>Trip1</td>
<td>6</td>
<td>0.113</td>
<td>0.043</td>
<td>0.094</td>
<td>0.026</td>
<td>0.103</td>
<td>0.031</td>
</tr>
<tr>
<td>Stanton</td>
<td>2</td>
<td>0.133</td>
<td>0.064</td>
<td>0.109</td>
<td>0.033</td>
<td>0.145</td>
<td>0.059</td>
</tr>
<tr>
<td>Greasy</td>
<td>1</td>
<td>0.111</td>
<td>0.098</td>
<td>0.055</td>
<td>0.025</td>
<td>0.111</td>
<td>0.098</td>
</tr>
<tr>
<td>Sturgeon</td>
<td>5</td>
<td>0.121</td>
<td>0.054</td>
<td>0.095</td>
<td>0.029</td>
<td>0.106</td>
<td>0.036</td>
</tr>
</tbody>
</table>
Table 2.5. Pairwise $F_{ST}$ for all ten sampling sites.

<table>
<thead>
<tr>
<th></th>
<th>LFal</th>
<th>SFL</th>
<th>Trip2</th>
<th>Craney</th>
<th>Trip1</th>
<th>RRG</th>
<th>Stanton</th>
<th>Greasy</th>
<th>Sturgeon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Konnick</td>
<td>0.078</td>
<td>0.121</td>
<td>0.104</td>
<td>0.121</td>
<td>0.118</td>
<td>0.121</td>
<td>0.161</td>
<td>0.209</td>
<td>0.177</td>
</tr>
<tr>
<td>LFal</td>
<td>0.068</td>
<td>0.061</td>
<td>0.071</td>
<td>0.071</td>
<td>0.087</td>
<td>0.112</td>
<td>0.139</td>
<td>0.133</td>
<td></td>
</tr>
<tr>
<td>SFL</td>
<td>0.104</td>
<td>0.128</td>
<td>0.114</td>
<td>0.120</td>
<td>0.166</td>
<td>0.226</td>
<td>0.181</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trip2</td>
<td>0.082</td>
<td>0.065</td>
<td>0.111</td>
<td>0.149</td>
<td>0.196</td>
<td>0.167</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Craney</td>
<td>0.094</td>
<td>0.124</td>
<td>0.182</td>
<td>0.264</td>
<td>0.192</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trip1</td>
<td>0.123</td>
<td>0.164</td>
<td>0.212</td>
<td>0.181</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RRG</td>
<td>0.099</td>
<td>0.139</td>
<td>0.124</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stanton</td>
<td></td>
<td>0.240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greasy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.211</td>
</tr>
</tbody>
</table>
Table 2.6. Tajima’s D at multiple scales including sites, basins, population clusters, and all together.

<table>
<thead>
<tr>
<th></th>
<th>Konnick</th>
<th>LFal</th>
<th>SFL</th>
<th>Trip2</th>
<th>Trip1</th>
<th>RRG</th>
<th>Sturgeon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SITES</strong></td>
<td>0.125</td>
<td>-0.180</td>
<td>-0.095</td>
<td>-0.046</td>
<td>0.303</td>
<td>-0.072</td>
<td>0.260</td>
</tr>
<tr>
<td><strong>BASINS</strong></td>
<td>Kinniconick</td>
<td>Licking</td>
<td>Kentucky</td>
<td>0.125</td>
<td>-0.380</td>
<td>-0.225</td>
<td></td>
</tr>
<tr>
<td><strong>CLUSTERS</strong></td>
<td>Kinniconick/Licking</td>
<td>Kentucky</td>
<td>-0.445</td>
<td>-0.225</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ALL</strong></td>
<td>-0.787</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.7. AMOVA results for A) Populations according to Basin (3 Populations) and B) Populations according to Likelihood analyses (2 Populations).

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>df</th>
<th>Variance components</th>
<th>Percentage of variation</th>
<th>Fixation indices</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Among Basins</td>
<td>2</td>
<td>0.854</td>
<td>2.953</td>
<td>0.030</td>
<td>0.145</td>
</tr>
<tr>
<td>Among sites within basins</td>
<td>7</td>
<td>6.033</td>
<td>11.799</td>
<td>0.122</td>
<td>0.001</td>
</tr>
<tr>
<td>Among samples within sites</td>
<td>31</td>
<td>-9.316</td>
<td>85.248</td>
<td>0.148</td>
<td>0.001</td>
</tr>
<tr>
<td>B) Among Basins</td>
<td>1</td>
<td>3.364</td>
<td>6.745</td>
<td>0.067</td>
<td>0.018</td>
</tr>
<tr>
<td>Among sites within basins</td>
<td>8</td>
<td>6.095</td>
<td>10.157</td>
<td>0.109</td>
<td>0.001</td>
</tr>
<tr>
<td>Among samples within sites</td>
<td>31</td>
<td>-9.475</td>
<td>83.098</td>
<td>0.169</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Figure 2.1. Location of sampling sites (triangles) and dams (black circles) in Kentucky. Sampling sites are numbered 1-10 with full details for each provided in Table 2.1. From Northeast to Southwest, there is the Kinniconick basin with site 1 (Yellow), the Licking Basin with sites 2-6 (Red), and the Kentucky basin with sites 7-10 (Green).
Figure 2.2. Plot of cross-validation errors for ADMIXTURE K selection. A lower error corresponds to an increased likelihood of that number of populations.
Figure 2.3. Bar plot with the admixture estimates for A) K=2, B) K=3, C) K=4, and D) K=5. In A, we see a separation of the Kentucky and combined Licking/Kinniconick basins. In B, the Sturgeon creek site separates. In C, Kinniconick creek separates. In D, the combined South Fork Licking Site and the main stem Licking Site separate.
Figure 2.4. PC plot for two populations found using the find.clusters function in adegenet using Discriminant Function 1 on the x-axis.
### Written by Paul Hime.
### This is a script to stitch ddRAD reads together. 
### It will reverse complement the sequences and reverse the qualities in R2. 
### It then pastes (stitches) R1 and R2 together into a fastq file for input into Stacks. 
### 
### Modify the paths to fastq files on line 16 and 20, and the output file path on line 32

```
echo -e "\nTIME SPENT READING R1:"
time cp /home/mmu235/catlibs/Nect_R1.fastq R1 ### replace /home/pmhi222/ddRAD_v3/R1.fastq with your R1 file
echo -e "\nTIME SPENT READING R2:"
time cp /home/mmu235/catlibs/Nect_R2.fastq R2 ### replace /home/pmhi222/ddRAD_v3/R2.fastq with your R2 file
echo -e "\nTIME SPENT REVERSE COMPLEMENTING R2 SEQUENCES:"
time awk 'NR%4==2' R2 | awk '{ print "\n\n"$1;}' | sed -e '1,2d' | rev | tr ACGT TGCA > R2.seq.rc

echo -e "\nTIME SPENT REVERSING R2 QUALITY SCORES:"
time awk 'NR%4==0' R2 | awk '{ print "\n\n"$1;}' | rev > R2.qual

echo -e "\nTIME SPENT STITCHING R1 TO R2:"
time paste R1 R2.seq.rc R2.qual | awk '{gsub("\t","",$0); print $0;}' | awk '{gsub(" 1:N:0:"," stitched:N:0:"),$0); print $0;}' > /home/mmu235/catlibs/R1_R2.stitched.fastq

echo -e "\nTIME SPENT CLEANING UP:"
time rm R2.seq.rc R2.qual R1 R2

echo -e "\nALL DONE"
```
REFERENCES


Institution Press, Washington DC, USA.


VITA

NAME: Mason Owen Murphy

EDUCATION: B.S., Biology, 2013
University of Notre Dame
Notre Dame, IN, USA