



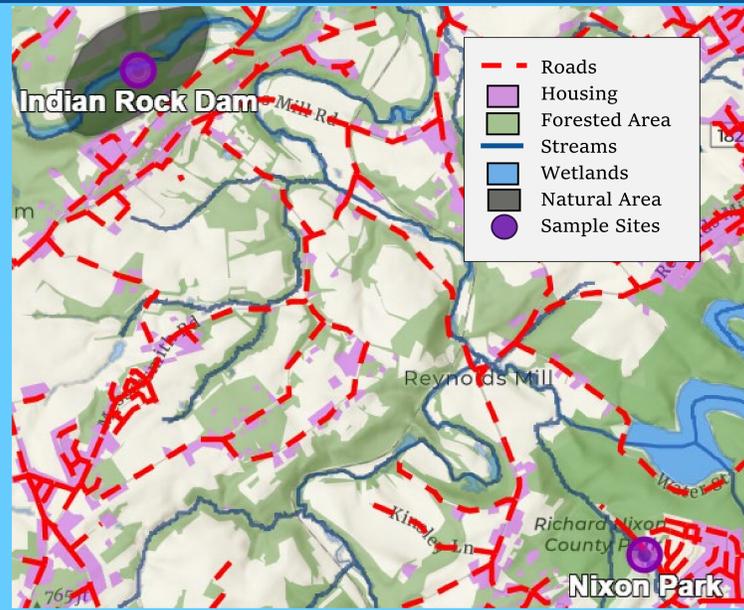
# Leap into Conservation

## Comparing Genetic Variation between two Populations of Spring Peepers (*Pseudacris crucifer*)

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### Background

- Amphibians are experiencing a devastating decline, partially as a response to fragmentation and low connectivity among populations (Cushman, 2006).
- Genetic variation is an indicator of populations persistence. High genetic variation is maintained by established connectivity between populations (Grant et. al, 2016)
- Microsatellites, a non-coding sequence of DNA that has a 'Short Tandem Repeat' (i.e.,  $(GAA)_n$ ), are often used to measure genetic variation.
- Spring Peepers, a small chorus frog found across York County, PA in abundance during mating season in spring were easily accessed as a population model for amphibians (Lehtinen, & Galatowitsch, 2001).
- Currently it is unknown where the spring peepers observed in Nixon Park migrated from, as they were not always present



**Figure 1.** Map indicating the two labeled site locations (purple), wetlands, streams (blue), forested areas (green) natural areas (gray), roads (red) and building data (pink) throughout the study area, York County, PA.

**Objective:** To quantify and observe the genetic differences between two locations of Spring Peepers (*Pseudacris crucifer*) in order to understand their connectivity.

**Hypothesis:** Genetic differentiation among the two breeding locations will be high due to low connectivity and high fragmentation of the environment.

#### Acknowledgements

Thank you to my mentor Dr. Bridgette Hagerty for assisting with all aspects of data collection & analysis and to Dr. Stephen Beck for helping to acquire and map GIS data. I would also like to thank York County Parks for granting permission to collect samples in the wetlands. All research activities in this study were approved by the York College of Pennsylvania Institutional Animal Care and Use Committee.

### Optimization of Microsatellites

(Degner et al., 2008)  
(Stewart et al., 2015)

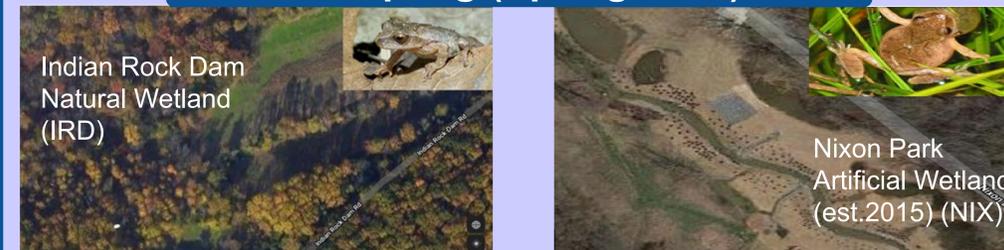
Pcru09 Pcru10 Pcru14  
Pcru24 Pcru32 Pcru05  
Pcru11 Pcru21 Pcru06  
Pcru12 Pcru08



Pcru14 (184bp-232bp)  
Pcru06 (186bp-236bp)  
Pcru08 (222bp-270bp)

Primers that amplified consistently in multiple individuals were chosen for analysis

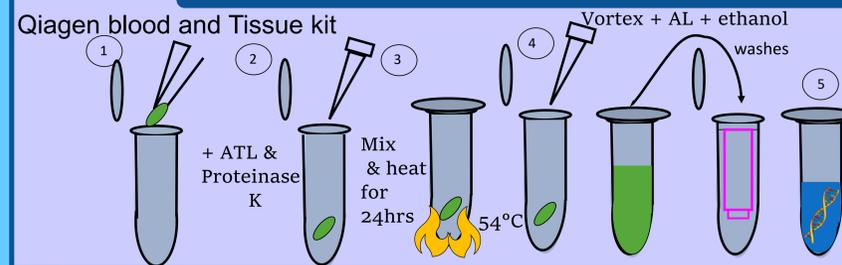
### Sampling (Spring 2019)



**Table 1.** Number of individuals collected, Average Temperatures (°C) and Average lengths of individuals collected throughout sampling events across each breeding pool location.

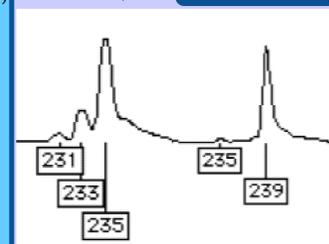
Site	# of Individuals Collected	Average Temperature (°C)	Average Length (mm)
NIX	18	13.5	24.90
IRD	26	17.5	25.30

### DNA Extraction



(Peakall & Smouse, 2012)

### Analysis of Population Genetics



- Identification of allele size through analysis of fluorescently labeled microsatellites to create genotypes.
  - Genotypes of each individual (n=32\*) was analyzed to determine allele frequencies and other population genetic values, calculated using GenAlix in Excel
- \*Nixon Park (n=15) & Indian Rock Dam (n=17)

#### Citations

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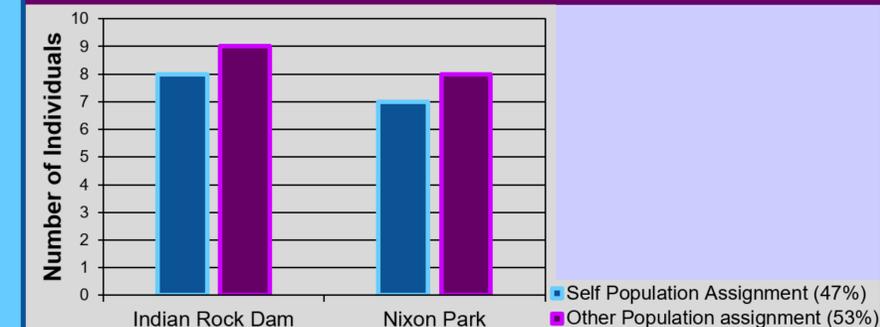
### Results

**Table 3.** Population data including, Means  $\pm$  standard deviation, Number of alleles (Na), Observed Heterozygosity (Ho), Expected Heterozygosity (He), Unbiased Expected Heterozygosity (uHe) and Fixation Index (F) for Richard Nixon Park (NIX) and Indian Rock Dam, New Salem (IRD). Data was analyzed from GenAlix v.6.503 add in, (Peakall & Smouse, 2012).

	Na		Ho		He		F	
	IRD	NIX	IRD	NIX	IRD	NIX	IRD	NIX
Mean	8.33	8.00	0.62	0.72	0.81	0.78	$\pm 0.06$	$\pm 0.12$
$\pm$ SD	$\pm 0.88$	$\pm 1$	$\pm 0.02$	$\pm 0.07$	$\pm 0.04$	$\pm 0.03$		

#### Number of Alleles, He & Ho & F

- Na is a moderate value for both locations
- Expected heterozygosity (He) is higher than observed heterozygosity for both locations. IRD had lower He & Ho. Findings are suggestive of random mating, as they are not close in value
- F was higher for IRD, suggesting less individuals live in this location. This was not the case, indicating individuals alleles may have migrated between both locations or other close locations
- Differentiation was reported as a pairwise  $F_{ST}$  value, 0.022, suggesting no difference in alleles



**Figure 2.** Population Assignment test output conducted on individuals collected from each breeding pool location, Nixon Park (n = 15) and Indian Rock Dam, New Salem (n= 17). All data was conducted in and obtained from GenAlix v.6.503 add in, (Peakall & Smouse, 2012).

#### Connectivity is occurring

- Approximately half of the individuals assigned to its location of origin (self), indicating the breeding locations have shared allele frequencies.

### Conclusions

- Allele frequencies resulted in low levels of genetic assignment to the breeding locations as well as low  $F_{ST}$  value. This suggests that connectivity is present between breeding locations with a low possibility of inbreeding and high probability that migrants are being exchanged.
- Colonization of the Nixon Park wetland may include individuals from the same population as Indian Rock Dam vernal pool. These results may also suggest a possibility of metapopulations existing within the span of the study area.

#### Future Studies

- Increasing the number of locations sampled to delineate the presence of potential metapopulations.
- Optimizing more primers, adding more loci to increase power.