

## Using the Eastern Hellbender Salamander in a High School Genetics & Ecological Conservation Activity

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### ABSTRACT

This article contains an original 5E lesson plan developed from conservation genetics research on the giant North American hellbender salamander, *Cryptobranchus alleganiensis alleganiensis*. The lesson plan provides background information on the hellbender, reviews basic genetics, and exposes students to the scientific process that is used during PCR (polymerase chain reaction) and the analysis of microsatellites in order to calculate allele and genotype frequencies for understanding genetic variability. Once completing the lesson, students should make the connection between molecular research and conservation of a rare, cryptic, and interesting species.

**Key Words:** Genetics; PCR; gel electrophoresis; conservation genetics; hellbender salamander; biodiversity.

Teachers face many challenges in today's high school science classrooms. Many students are disengaged, find science difficult, or fail to see science as having any relevance in their lives (Logan & Skamp, 2008). Because of this, it is of paramount importance that teachers develop lessons to engage students and that help them realize the importance of science and its impacts on society. Current applications commonly used with the standard high genetics unit include crime-scene investigation

and paternity testing. This pencil-and-paper activity provides conservation genetics as a new application teachers can use with the genetics unit.

The following high school biology or environmental science lesson and activity were developed on the basis of a graduate research project conducted by the first author on the conservation genetics of eastern hellbender salamanders (*Cryptobranchus alleganiensis alleganiensis*). Recognizing an opportunity to translate and apply authentic data from a scientific research study, the development of a lesson and activity followed, which were tested in a variety of

*Teachers face many challenges in today's high school science classrooms.*

classroom settings at both the high school and college levels. The lesson and activity are designed to

- provide students with a real-life application of genetics,
- expose students to a species they most likely do not know exists, and
- increase students' awareness of how genetics research plays an important role in the conservation of a rare species.

Conservation genetics combines principles of ecology and evolution with molecular techniques and mathematical modeling to determine the genetic structure and diversity of rare or endangered organisms. Conservation genetics is used as a way to measure and monitor the genetic diversity of species present in various ecosystems, which can then be used in a management plan to help maintain or restore appropriate levels of biodiversity. High levels of biodiversity are correlated with stable ecosystems. In addition, the conservation of genetic diversity is important to help a species survive through natural selection.

This activity introduces students to a rare and interesting amphibian, provides a basis for conservation genetics, and promotes active engagement and analytical thinking about how molecular population genetics is accomplished.

It also incorporates many of the tenets of the nature of science: scientific knowledge is tentative; science is empirically based on or derived from observations of the natural world; and science is inferential, imaginative, and creative, and is a human endeavor.

### ◎ The Hellbender Salamander

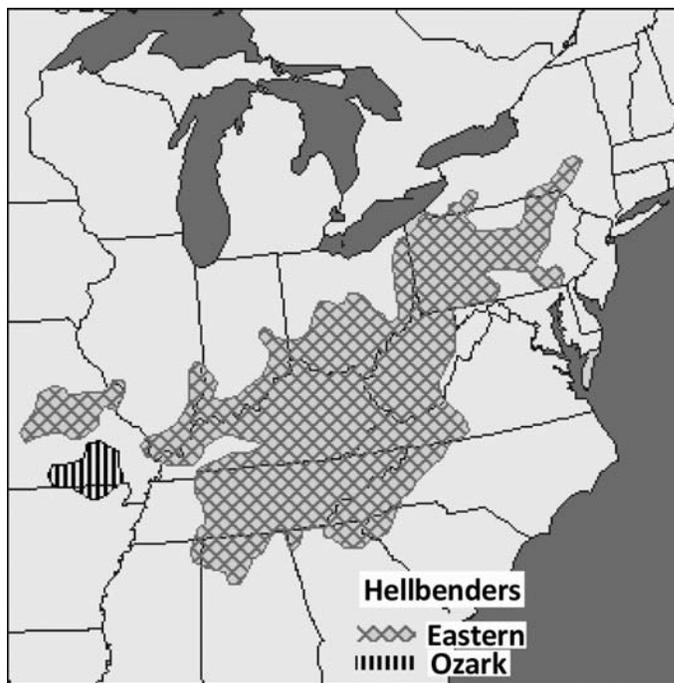
We are living in a time of rapid declines in many species, some of which have resulted in extinction. Populations of the eastern hellbender salamander are quickly declining, and the species is

classified as “near threatened” on the IUCN (International Union for the Conservation of Nature) Red List of Near Threatened Species, and is under consideration for the U.S. Fish and Wildlife Service Endangered Species list. Factors contributing to their decline may include predation, new diseases, changes in climate, and, probably most importantly, humans illegally collecting them or modifying their natural aquatic habitats with dams, agricultural and construction sedimentation, and pollution.

Hellbenders are giant aquatic salamanders that live all of their life stages in water. They grow to an average length of 50 cm, with some up to 74 cm (>2 feet long; Figure 1). The eastern hellbender is found in a number of the eastern states, with the northernmost range in southern New York. Its range continues south and west through states such as Georgia, Alabama, and Missouri (Nickerson & Mays, 1973; Figure 2).

Special characteristics of the hellbender include a large flat head, wide neck, dorsoventrally flattened body that is heavily wrinkled, and a keeled tail (Green & Pauley, 1987; Figure 3). Hellbenders have external gills as larvae, and these gills are lost during metamorphosis. After this metamorphosis, hellbenders have to be able to absorb

oxygen from the water through the folds in their skin, so they need to live in streams with fast-moving, oxygenated water (Bishop, 1941). Even though hellbenders breathe through their skin, they have lungs, which makes their respiratory system unique (Guimond, 1970).



**Figure 2.** Hellbender habitat range (courtesy of the New York State Department of Environmental Conservation, <http://www.dec.ny.gov/animals/7160.html>).



**Figure 1.** Meghan Jensen, SUNY Buffalo State graduate student, holding an adult hellbender salamander.



**Figure 3.** Adult hellbender in its natural aquatic habitat (photo by Alysha Trexler, used with permission; courtesy of the Western Pennsylvania Conservatory, <http://www.paconserve.org>).

Hellbenders play an important ecological role as both predator and prey. The predators of hellbenders consist of various fishes and mammals. Hellbenders prey upon crayfish and some small fish and aquatic insects.

## 📍 Conservation Genetics

In order to help declining populations of hellbenders, scientists need to figure out how best to conserve them (see Help the Hellbender, Purdue Extension, <http://www3.ag.purdue.edu/extension/hellbender/Pages/default.aspx>). Conservation biologists are concerned with preserving biodiversity in natural areas, and so understanding the population structure of hellbenders helps researchers determine which populations are stable and diverse and which may need management. Genetic markers such as microsatellites can be used to study the population structure. A microsatellite is a segment of DNA that has a repeating set of nucleotides (such as the sequence CAGA repeated eight times). Because microsatellites have high mutation rates, they are a useful tool for measuring genetic variation in a population. Microsatellites are found in noncoding regions of DNA but are indicators of genetic variability in DNA regions that do code for genes. High microsatellite variability may indicate more overall genetic variation in the population, which will help a population better survive through natural selection.

Microsatellites are used to determine the genotype of each hellbender. Collectively, all of the genotypes are used to determine how much genetic variation is present in the population. Polymerase chain reaction (PCR) is used to make many copies of the microsatellite regions of DNA (this is called “DNA amplification”). In the eastern hellbender, 12 microsatellite regions have been identified, and 10 are useful for genotyping, since they show a lot of genetic variability. In order to amplify the microsatellite region, a primer (a DNA sequence that is required for starting DNA replication) specific to the microsatellite sequence must be used. The technique of PCR uses a thermal cycling machine, which brings the DNA through several stages of heating and cooling. There are three main thermal phases that take place during PCR. First, the double-stranded DNA is heated and therefore denatured to separate the DNA into two separate strands. Next the temperature is lowered so that the primers can anneal (or attach) to the DNA strands only in the microsatellite region of interest. Finally, the temperature is raised so that the enzyme DNA polymerase can elongate (synthesize or build) a new copy of each DNA strand of the specific microsatellite. Billions of copies of each specific microsatellite are made after 30 cycles.

After DNA amplification, there are enough copies of the microsatellite DNA to genotype the hellbenders. The technique used for genotyping is gel electrophoresis. In this case, hellbender DNA samples are loaded into a polyacrylamide gel, and an electrical current is sent through the gel to separate the DNA fragments by size. The gel is then stained so that the DNA size fragments (bands) can be visualized. The bands that form in the expected size range of the microsatellite correspond to the genotype for that hellbender. To speed up the process of

genotyping, a fluorescent detection machine is used instead of gel electrophoresis. During PCR, a fluorescent dye is added that is incorporated into the DNA. After PCR, the amplified DNA is separated with electrophoresis in a fluorescent detection machine, and the genotype of the hellbender is determined from the fluorescent peaks that form (see below, Figure 7). Researchers use several different microsatellite sequences for genotyping to most accurately determine the amount of genetic variation present in the population.

Conservation genetic work has been done on many hellbender populations. For example, the microsatellite locus used in this activity has many alleles. One of the most common alleles is 205 (this allele is 205 base pairs long when amplified with the primers shown in this activity). In the main river in the New York Allegheny drainage, allele 205 occurs 28% of the time. However, it is only 12% and 7% of the alleles in two of the tributaries. In a Pennsylvania tributary it occurs 21% of the time, and in a group of hellbenders being used to repopulate the streams it is 14% of the alleles at this locus. Also, researchers have discovered that hellbenders from different river systems throughout their range are genetically different (Tonione et al., 2011). As a result, these systems should be managed independently, rather than as one big population. Work on the genetic diversity of hellbenders in the Allegheny and Susquehanna Rivers in New York and Pennsylvania is ongoing because these populations have declined and repopulation efforts are underway (Jensen, 2013).

All of the classroom materials for students and teachers described in this article, including the PowerPoints, answer keys, and additional hellbender resources, can be found at <https://sites.google.com/site/sachudyknysbiology/hellbender-salamander-conservation-genetics>.

## 📍 Classroom Activity: Hellbender Conservation Genetics

The student learning outcomes for this activity have been correlated to the *Next Generation Science Standards* (Table 1).

### Materials

(All activity materials are found on website.)

- Projector or SMART board
- PowerPoint presentation

**Table 1. Student learning outcomes and correlation to standards.**

Student Learning Outcomes	Next Generation Science Standards
Model the amplification of DNA during PCR	HS-LS3-1
Identify a microsatellite region in a sequence of DNA	HS-LS3-1
Identify primers in a sequence of DNA	HS-LS3-1
Identify bands in a gel as either homozygous or heterozygous	HS-LS3-1, 2
Determine genotypes of bands on a gel	HS-LS3-1, 2
Calculate and graph allele and genotype frequencies	HS-LS3-3; HS-LS4-3
Determine genetic variability from allele and genotype frequency graphs	HS-LS3-3; HS-LS4-3
Explain the role of genetics in the conservation of the eastern hellbender salamander	HS-LS2-6, 7 HS-LS4-3, 4, 5, 6

- Guided notes
- Templates and student activity guide
- Additional hellbender resources

## Overview of Lesson Components

- Engagement (Fact vs. Myth & YouTube)
- Exploration
  - # Step 1: PowerPoint
  - # Step 2: Modeling PCR
  - # Step 3: Microsatellites
  - # Step 4: Gel Electrophoresis
  - # Step 5: Genotype Exercise
- Elaboration: Fluorescent Detection
- Explanation: Allele and Genotype Frequencies
- Evaluation

## Procedure

### Engagement: Background (15–20 minutes)

- (1) “Fact or myth” (on website)
- (2) YouTube Video “Hellbender! the latest Zoo Animal Misconception” (<http://www.youtube.com/watch?v=5sz3LoqPSwQ>)
- (3) Students self-correct “Fact vs. Myth”
- (4) PowerPoint “What We Know about Hellbenders”

### Exploration: Conservation Genetics (60–75 minutes)

**Step 1:** PowerPoint “Hellbender Conservation Genetics” (on website)

**Step 2:** Modeling PCR (on website). Using an eight-base-pair DNA template provided on the website, students will replicate the parent strand into daughter and third-generation strands.

**Step 3:** Microsatellites. *Note:* Forward and reverse primers are needed to amplify the microsatellite region during PCR. On Figure 4, the forward primer is the light gray highlighted sequence in front of the microsatellite (dark gray), and the reverse primer is after the microsatellite.

Using the student activity “Microsatellites” located on the website and the DNA sequence in Figure 4, students will

- (1) Identify the microsatellite sequence using the correct nomenclature and identify the forward and reverse primer sequences, which are highlighted in the hellbender DNA sequence (Figure 4).
- (2) Create the entire microsatellite sequence  $[GATA]_8$  identified from the hellbender DNA sequence and show what the new strand of DNA would look like when it is replicated during PCR.

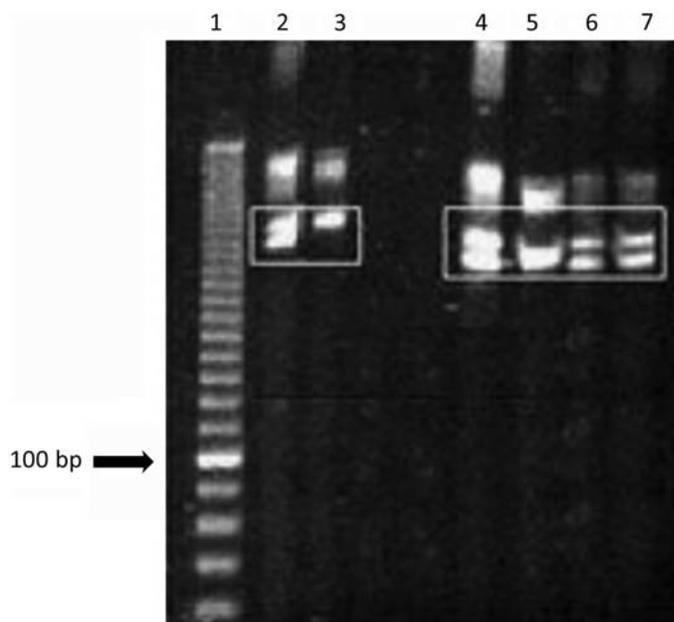
**Step 4:** Gel Electrophoresis Exercise. On Figure 5, lane 1 contains a 10-base-pair DNA ladder, and the arrow indicates a DNA fragment that is 100 base pairs in size. Each “rung” of the ladder is a 10-base-pair interval, with larger fragments above the arrow and smaller fragments below the arrow.

Using the student activity “Gel Electrophoresis” on the website and the actual data in Figure 5, students will identify the number of

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accataatg gtaatagctg catggaataa aagactaatt agatagatag atagatagat
agatagatag atagatagat agatagatag atagatagat agatagatag atagagacat
tcaaatgcct cagtgggtga aacacagtag aggaagttaa catatttcag cggaggtgtg
aatctggaac aagggtccat gagataagat ttgaaggggg gaggctgagg agtaacatga
ggaaat
  
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**Figure 4.** Partial hellbender single-strand DNA sequence with microsatellite (dark gray) and primer (light gray) sequences highlighted (Chudyk, 2013).



**Figure 5.** Picture of actual hellbender gel showing six samples with band formation (Chudyk, 2013).

bands present in each lane and identify these as either homozygotes or heterozygotes (Figure 5). If the hellbender is homozygous, one band will form. If the hellbender is heterozygous, two bands will form.

A depiction of a hellbender gel has been created (see Figure 6) from the gel in Figure 5 in order for students to understand how scientists use the data obtained from gel electrophoresis to determine the genotype of the DNA samples under investigation.

**Step 5:** Genotype Exercise. Using the student activity “Genotype Activity” (Figure 6) also found on the website, students will identify the genotypes for each hellbender by completing the table that represents 10 real hellbender DNA samples. The genotype 213/225 is shown (see column 1 on Figure 6) and corresponds to the size of the microsatellite fragments on the gel. The numbers on the DNA ladder correspond to the sizes of the DNA fragments in base pairs and are used as the allele numbers to identify the genotype of each hellbender. After determining the genotypes of the hellbenders, the genotype and allele frequencies can be calculated to determine the amount of genetic variation in the population.

### Elaboration: Fluorescent Detection (5 minutes)

During scientific research, genotyping on a gel is much more difficult and would take much longer. To speed up the process, a fluorescent detection machine is used to determine the genotypes. Using the genotyping results produced from a fluorescent detection machine used during this research, students will identify which

peaks represent the DNA ladder and which peaks show the genotype of the hellbender (Figure 7). The short peaks (labeled 180, 190, 200, 220, and 240) represent the DNA ladder. The tall peaks are the allele peaks, which correspond to this hellbender's genotype (202/218). The x-axis shows the size of the DNA fragments in nucleotides (nt), equivalent to base pair sizes. The y-axis shows the strength of the fluorescence in relative fluorescent units (RFU).

### Explanation (15–20 minutes)

The final aspect of this activity is a two-part process that instructs students to calculate the frequency of the occurrence of the alleles, followed by a final step, which is to calculate the number of hellbenders with each genotype.

**Step 1:** The following chart (Table 2) lists the alleles present at one microsatellite region for a sample of 25 hellbenders from one population. Also listed is the number of times that allele appears in the population. Students will calculate the allele frequency using the formula below. The first allele has been completed as an example.

Frequency =

$$\frac{\text{number of specific allele in population}}{\text{total number of alleles in population}} \times 100 = \text{_____}\%$$

Students can use the data to make a bar graph (or histogram). On the graph, label the x-axis with the allele numbers (from the first column on the chart) and label the y-axis as frequency (%).

**Step 2:** Table 3 shows the genotypes that are present at one microsatellite region for the same sample of 25 hellbenders from one population. Also listed is the number of times that genotype appears in the population. Calculate the genotype

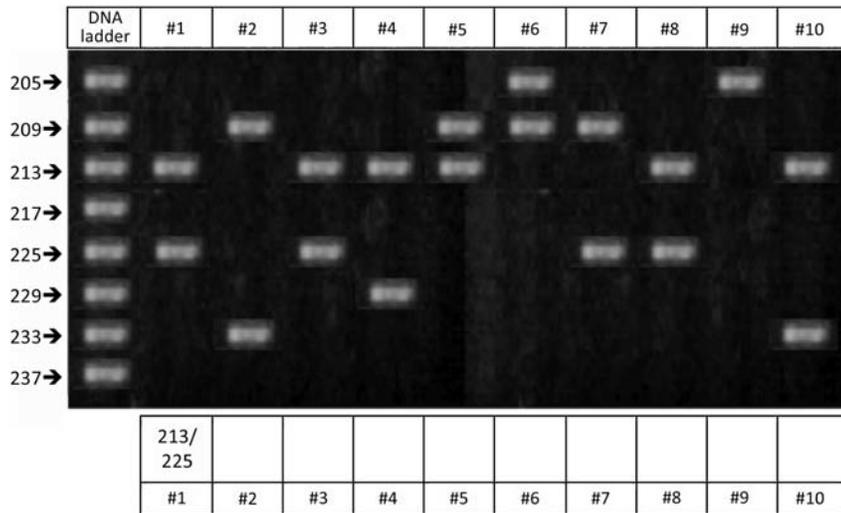


Figure 6. Representation of hellbender genotypes.

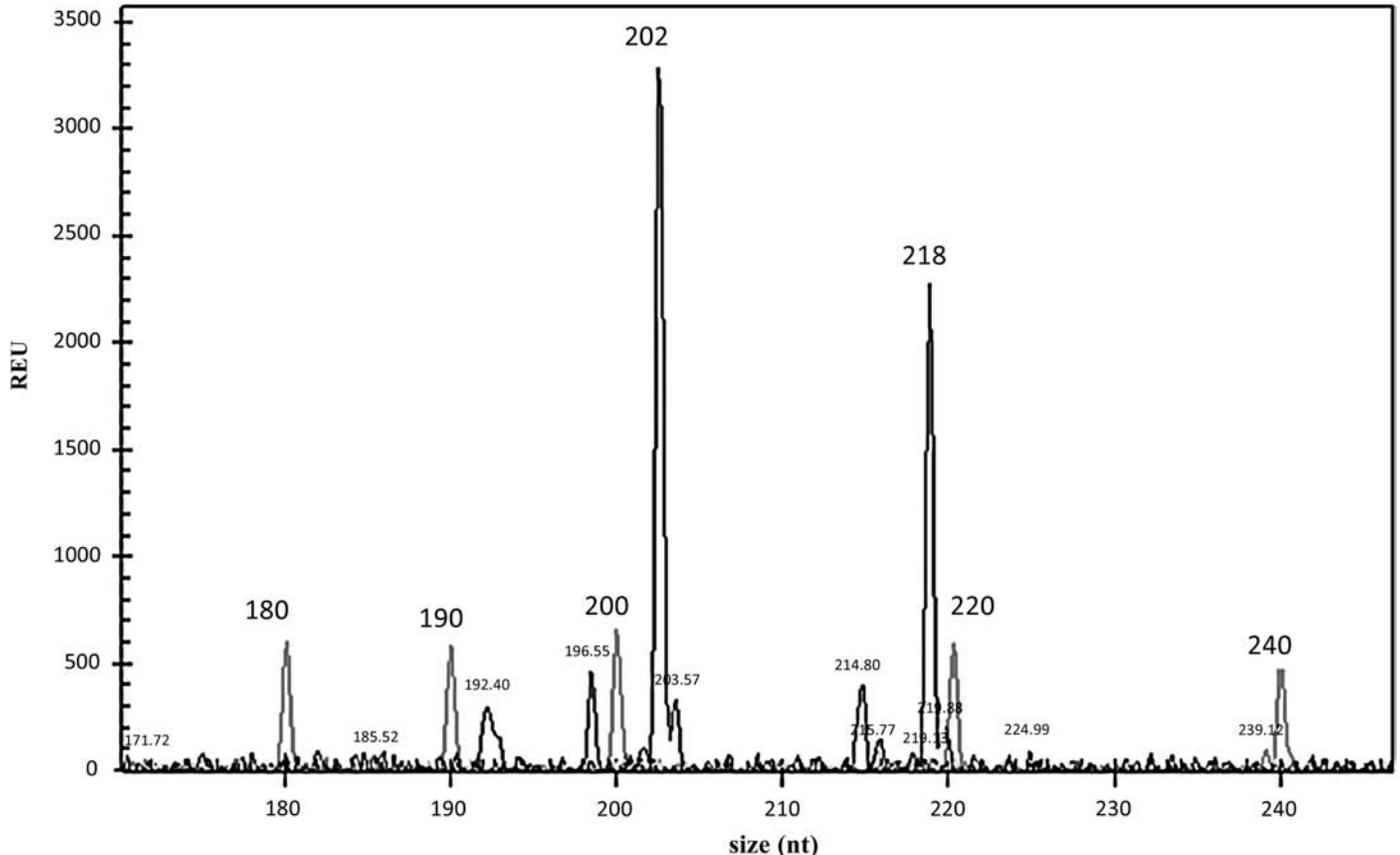


Figure 7. Genotyping results from a fluorescent detection machine (Chudyk, 2013).

**Table 2. Data table for determining the frequency of alleles.**

Allele	Number of Alleles Present (Total = 50)	Frequency (Show Calculations)	Frequency (Final Answer as a Percentage)
205	8	$8/50 = 0.16 \times 100 = 16\%$	16%
209	12		
213	12		
217	1		
225	9		
229	4		
233	4		

**Table 3. Data table for determining the frequency of genotypes.**

Genotype	Number of Hellbenders with this Genotype (Total = 25)	Frequency (Show Calculations)	Frequency (Final Answer as a Percentage)
205/205	1	$1/25 = 0.04 \times 100 = 4\%$	4%
205/209	1		
205/213	2		
205/217	1		
205/229	2		
209/209	1		
209/213	2		
209/225	3		
209/229	1		
209/233	3		
213/225	6		
213/229	1		
213/233	1		

frequency using the same formula, but replace alleles with genotypes. The first genotype has been completed as an example.

Students can use the data to make a bar graph (or histogram). On the graph, label the x-axis with the genotype (from the first column on the chart) and label the y-axis as frequency (%).

### Evaluation (15–20 minutes)

Students will answer the following concluding questions to assess their knowledge and understanding of this activity. Teachers could have students answer these questions as an in-class activity or complete them for homework.

- What can be done to help us figure out how best to conserve hellbenders?
- What type of genetic markers can be used to study population structure? Explain what these markers are and why they are useful.

- Mutations are often harmful to a species. However, mutations can sometimes be beneficial. Why can high mutation rates be a good thing?
- DNA is amplified through the use of PCR. What is specifically needed to amplify a microsatellite region?
- What are some observations/conclusions you can make from the allele frequency data? What does this show about genetic variation in these hellbenders?
- What are some observations/conclusions you can make from the genotype frequency data? What does this show about genetic variation in these hellbenders?
- What are some overall conclusions you can make from this activity? Make sure to include why all of this is important – Why do we care?

### Conclusion

This conservation genetics activity should be an excellent activity for high school teachers to use with their biology classes. This activity uses authentic data that was collected for an actual study and describes methods used by conservation geneticists in order to demonstrate and provide investigative resources. Students will learn about the importance of conservation in a unique and rare species. Students also will gain an understanding of how genetics can be used to study populations to help maintain high levels of biodiversity and stable ecosystems.

### Acknowledgments

We thank the New York State Department of Environmental Conservation, Buffalo Zoo, SUNY Buffalo State, AZA Amphibian Taxon Advisory Group Conservation Fund, U.S. Fish and Wildlife Service, and Tim Fedele for contributions made to this research on hellbenders. We thank two anonymous reviewers and Michele Parente, whose comments and suggestions helped improve and clarify the manuscript.

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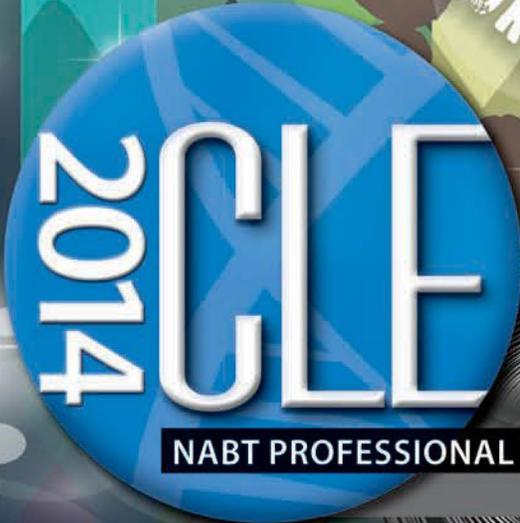
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