Cutthroat trout in Colorado: A case study connecting evolution and conservation

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Abstract

Evaluation of evidence is a key process skill for the core competencies of applying the process of science and using quantitative reasoning. This case study enables upper-division biology students to practice these skills by applying evolutionary concepts to a real-world conservation problem and make evidence-based decisions accounting for uncertainty in real data sets. Cutthroat trout have a long evolutionary history of allopatric speciation and a complicated history of movement by humans for subsistence, recreation, and conservation purposes. This case study engages students because it requires a fundamental understanding of evolutionary processes such as speciation and hybridization and raises questions about the value of native species and the goals of conservation efforts. In an interrupted lecture format that can be adjusted for one longer 75- or 120-minute period or two shorter 50-minute periods, students learn about the Endangered Species Act, the evolutionary and human histories of cutthroat trout, and examine figures in order to make recommendations for the conservation of cutthroat trout. This case study shares primary research in the field of conservation genetics with students and allows them to grapple with the complexity of the decision-making process in wildlife management. Students enjoy connecting information that may be abstract for many (evolutionary processes and analyses) to a system (cutthroat trout) that they find tangible and relatable. This case study will be useful for courses in conservation biology, fish biology, and evolutionary biology, and adaptable for other contexts such as general biology and genetics.

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Supporting Materials: S1. Cutthroat Case Study Pre-Post Assessment Rubric, S2. Cutthroat Case Study Student Worksheet, S3. Cutthroat Case Study Lecture Slides, S4. Cutthroat Case Study Student Figures, S5. Cutthroat Case Study Worksheet Rubric, and S6. Cutthroat Case Study Worksheet Rubric.

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Learning Goal(s)

Students will:

- understand how the Endangered Species Act treats evolutionary processes
- connect evolutionary concepts to wildlife management practice
- understand how different kinds of data will lead to different conclusions

Learning Objective(s)

Students will be able to:

- interpret figures such as maps, phylogenies, STRUCTURE plots, and networks for species delimitation
- identify sources of uncertainty and disagreement in real data sets
- propose research to address or remedy uncertainty
- construct an evidence-based argument for the management of a rare taxon

INTRODUCTION

Origin of Lesson

"Cutthroat trout in Colorado" is an opportunity for upper-division biology students to apply evolutionary concepts to a real-world conservation problem. The main goal of the case study is for students to make evidence-based decisions accounting for uncertainty in real data sets. Accounting for uncertainty is a challenge for many students whose experience in science courses with canned data sets has led them to believe that there is a clear right answer to scientific questions. Our own experiences as conservation geneticists trying to piece together the evolutionary history of speciation and extinction and the human history of breeding and stocking led us to write this case study for our classrooms. The case of cutthroat trout in Colorado is appropriate because it requires a fundamental understanding of evolutionary processes such as speciation and hybridization and raises questions about the value of native species and the goals of conservation efforts. We wrote this case study to share primary research in the field of conservation genetics with students and to demonstrate the complexity of the decision-making process in wildlife management.

Context and Rationale

Evolution is poorly understood and even rejected by a large proportion of the American public: 60% of Americans accept the theory of evolution by natural selection, but only 32% ascribe it to natural causes (1,2). Evolution is one of the core concepts for undergraduate biological literacy identified by authors of Vision and Change (3). A strong understanding of evolution is essential to understanding biological systems at all levels, but students may struggle to relate the processes of natural selection and drift, speciation and hybridization, change and adaptation to conservation actions. Evolutionary theory is also at the core of conservation biology: conservation biologists and managers acknowledge the importance of population size and genetic variation in generating diversity and responding to changing conditions (4,5). Evolutionary concerns are also reflected in the designation of conservation units (6,7) and management plans that seek to conserve both process and pattern (8,9). As climate change and species loss continue in the face of expanding human populations, students who mature into citizens will be asked to make decisions about conservation actions; they will be better equipped to make these decisions with training in evolutionary biology and experience with the nuances of environmental decision making.

In addition, evaluation of evidence is a key process skill for the core competencies of applying the process of science and using quantitative reasoning (3). Students should be able to evaluate different kinds of evidence and identify the degree and potential sources of uncertainty in real data. Real data has error and uncertainty; how that uncertainty is handled during analysis and decision making has important real world implications(10). Different kinds of data and different analyses may lead to vastly different conclusions, in applications from climate modeling to taxonomic designations. Students need practice in confronting uncertainty to gain confidence and competence in how to handle uncertainty in different contexts. This case study asks students to look at data that were collected in different ways that may lead them to make different conclusions about the diversity of cutthroat trout in Colorado and to identify further research that would address the uncertainty in the available data.

Background

Cutthroat trout, *Oncorhynchus clarkii*, are an important target of conservation efforts in the American West. All recognized subspecies are currently under some form of state or federal protection (11). The cutthroat trout complex diversified during Pleistocene glaciation cycles, with lineages diverging in isolation in major drainage basins (12); the lineages are separated from one another by 300,000 to 3.6 million years of evolution (13,14) and from their sister species, rainbow trout, *Oncorhynchus mykiss*, by more than 10 million years (15). Colorado is currently home to four extant clades (an evolutionary lineage or group of organisms descended from a common ancestor) and was once home to at least six divergent lineages (16,17). However, until recently our understanding of the taxonomic diversity and biogeography of the clades was obscured by a long history of trout stocking without regard to lineage identity. Cutthroat trout, and many other fish, have been stocked in astounding numbers into populated and unpopulated waters throughout the American West for recreation, subsistence, and conservation purposes starting in the late 1800s and continuing to this day. As a result, the evolutionary history of cutthroat trout was overwritten by human stocking, with some species going extinct in competition with the introduced species and other species expanding their ranges as they were introduced into new drainages.

The greenback cutthroat trout, *O. c.* unnamed, is the Colorado state fish and was considered a major conservation success story. In the 1930s, it was declared extinct, likely due to overfishing, habitat loss from mining contamination, and the introduction of invasive salmonids such as rainbow and brook trout. In the 1950s, a group of graduate students on a camping trip in the mountains east of the Continental Divide in Colorado caught what they thought was the lost greenback, a "rediscovery" confirmed by morphological examination. Rediscovery prompted a search for remaining populations east of the Continental Divide, inclusion on the Endangered Species List in 1967, and major propagation and reintroduction efforts into eastern slope waters. By the early 2000s, the so-called greenback was established in many streams along the eastern slope and poised for delisting (18), the milestone for conservation success under the Endangered Species Act.

At the time delisting was being considered, the best understanding of the distribution of cutthroat trout diversity was that there were three extant lineages native to Colorado: the greenback east of the Continental Divide, the Colorado River cutthroat west of the Divide, and the Rio Grande cutthroat in southern Colorado. A fourth lineage from the headwaters of the Arkansas River was extinct. Conservation efforts were focused on increasing the size and number of greenback cutthroat populations east of the Divide. However, an investigation by University of Colorado scientists using molecular genetic data revealed that the cutthroat trout being called greenbacks were found on both sides of the Continental Divide and that many of the restoration populations were either hybridized or belonged to another lineage (16). This pattern is best explained by historical fish stocking, which did not discriminate among lineages. Because the uniqueness of the lineages was not yet recognized, the origin of stocked fish was not considered, resulting in mixing of lineages from both sides of the Continental Divide.

Genetic analysis of museum samples collected prior to the height of the fish stocking era revealed a more complex pattern of cutthroat trout diversity (17). Rather than four native lineages, Colorado was once home to six lineages, one in each of the major drainage basins: Green-Yampa, Colorado-Gunnison, San Juan, Rio Grande, Arkansas, and South Platte. The San Juan and Arkansas lineages are extinct (see Student Figure 6, on page 9). The Rio Grande lineage still persists in its native range, though populations in northern Colorado show evidence of hybridization due to stocking (19). The Colorado River cutthroat, thought to be native to the entire western slope, was actually native to the Green-Yampa drainage and had been stocked extensively on both sides of the Divide. What scientists and managers had been calling the greenback was actually native to the Colorado-Gunnison drainage west of the Continental Divide. And the true greenback, the lineage native to the South Platte drainage, was confined to a single population outside its native range (17). The lineage that had been stocked across the eastern slope was native to the Colorado-Gunnison drainage, and like the cutthroat from the Green-Yampa, had been extensively stocked across the state. The remnant population of true greenbacks is now being used for reintroduction efforts, but it is small and genetically depauperate. In this case study, students are asked to evaluate the evidence for native cutthroat trout diversity in Colorado and make an evidence-based decision about the future of this remnant population.

Intended Audience

This case study is intended for advanced biology students. This case study was implemented in Evolution, an upper division course that is required for the degree in Ecology and Evolutionary Biology at the University of Colorado. The majority of students in the course are sophomores and juniors; most are traditional students, with 5-15% non-traditional students, including older and returning students and veterans. The lesson was taught as part of both the regular semester course which averages 100-120 students and the summer course which averages 18-20 students. The case study works well as an application of micro- (gene flow and drift) and macroevolutionary (speciation and extinction) principles in a course focused on evolution but could be adapted for courses in conservation biology or conservation genetics.

Required Learning Time

In the large classroom, the lesson was taught over two 50-minute periods and included outside of class assignments (pre- and post-quizzes, 5 minutes each; homework assignment, 10-15 minutes). In the small classroom, it was taught over one 75-minute period and all work was completed in class.

Pre-requisite Student Knowledge

The lesson is framed by asking the students to think about conservation like evolutionary biologists. It assumes that they are comfortable reading phylogenies and maps and interpreting tables. It assumes they are familiar with species concepts; how gene flow, drift, and selection contribute to speciation; and how speciation is an on-going process. The skills required to interpret other kinds of figures commonly used in population genetics, such as a STRUCTURE plot and a haplotype network, are taught during the lesson. STRUCTURE is a program that assesses population genetic structure based on the genotypes of individuals; it is used to assess the probability that a particular individual belongs in a predefined group or to identify the number of populations or groups that best describes a set of individuals. Haplotype networks describe genetic similarity between single copy DNA such as mitochondrial haplotypes; unlike a phylogeny, it does not presume evolutionary relatedness. More information on both analyses is included below.

Pre-requisite Teacher Knowledge

Instructors should be comfortable with evolutionary concepts such as speciation, hybridization, gene flow, and genetic drift. They will need to be able to answer student questions about interpreting maps, tables, and figures including phylogenies, STRUCTURE plots, and haplotype networks. They may find it helpful to read or skim some of the primary literature from which the figures are taken, including (16) and (17). Students are provided with a worksheet that includes some information on the Endangered Species Act and cutthroat trout. Instructors should be able to expand that information slightly in their own introduction of the lesson, but expertise in cutthroat trout biology or the Endangered Species Act are not required to successfully teach the lesson. Additionally, when taught in a large classroom, instructors should be comfortable with managing discussion groups and asking for student responses.

SCIENTIFIC TEACHING THEMES

Active learning

Students engage in small-group and whole-class discussions to evaluate data and propose further research. Small group discussion is voluntarily reported to the whole class. New information is presented as part of an interrupted lecture to provide guidance and structure.

Assessment

- **Before class:** Students take a short multiple choice quiz online with 5 questions to assess their prior knowledge about hybridization, the Endangered Species Act, and interpreting figures. Suggested point value: 1 point per question for completion. (Supporting File 1: Rubric for pre-post assessment)
- **During class:** Students collaborate to interpret figures, evaluate data, and propose additional research. Suggested point value: 4 points per question for argumentation. (Supporting File 2: Student worksheet; Supporting File 6: Student worksheet key)
- **After class:** Students make recommendations based on the material in the case study and the course submitted online as free-response question and take a short multiple choice quiz with 5 questions that are isometric to the pre-test, to assess their learning gains. Suggested point value: 1 point per question for completion.

Inclusive teaching

- The lesson provides multiple types of data for students to explore and asks them to evaluate which kind they found most convincing. Additionally, they are asked to apply abstract knowledge about evolution to a real-world management scenario.
- Students collaborate with one another so that they may assist each other with figure interpretation, explain their thinking and strengthen their arguments, and modify ideas based on feedback.
- Cutthroat trout are a charismatic species that invite strong public engagement from anglers and conservationists. For students in the American West, cutthroat trout conservation is happening in their backyards and affects everything from recreational access to trails to water quality downstream.

LESSON PLAN

In different semesters, we implemented this lesson in either two shorter (50-minute) class periods or a single longer (75 or 120-minute) class period. Table 1 (starting on page 12) describes a lesson timeline that can be adjusted for shorter or longer time constraints with suggested time allotments for each part of the lesson. Based on our experience teaching this lesson, we have built flexibility into the time allotment for each part of the lesson. In the larger classroom (100-120 student lecture), the lesson was taught over two sessions, using about half of the second session for lesson material. The larger classroom required more time for transitions and discussing student feedback. In the smaller classroom (18-20 student lecture or lab), the lesson was taught in a single 120-minute session, including time for completing the homework assignment. Plan on adjusting the timing to meet the needs of your students and your own teaching style, keeping in mind that the active learning components should be the majority of the students' time.

Before Class Meeting

Instructor Preparation

We developed a short set of lesson slides to introduce and guide the lesson (Supporting File 3). To prepare for the lesson, you may download and modify the slides to suit your needs and style. You should also print out a copy of the student worksheet for each student (Supporting File 2); these sheets may be submitted individually or by group with each group member signing their name. Each group will need a copy of the figures (Supporting File 4); these figures reproduce moderately well in black and white, so you may find it helpful to provide a set of color copies for every two or three groups. If you use course management software (CMS), you can post the worksheet and figures so that students may access them electronically during the lesson. Pre-assessment questions are available (Supporting File 1) and can be distributed on paper, by clicker, or through your CMS. If you use the CMS, remind your students to take the quiz prior to coming to class; otherwise, allot 5 minutes prior to the lesson to administer the quiz. If your students do not frequently complete group work or do not have assigned groups, you may wish to inform them ahead of time that they will be working collaboratively and receiving credit for participation. Finally, you may find it helpful to read or skim the primary literature from which the lesson was developed (Metcalf et al. 2007, 2012).

Student Preparation

Students should take a short pre-assessment prior to or at the start of class. If you post the worksheet ahead of time, remind the students that they can familiarize themselves with the content, but should plan on collaborating with their peers on the answers during class time. You may also wish to have the students read about the Endangered Species Act; appropriate background material can be found on websites such as that of the United States Fish & Wildlife Service, the federal agency responsible for managing endangered species (http://www.fws.gov/endangered/laws-policies/).

During Class Meeting

Introduce the Lesson and the System

Begin the lesson by describing the learning goals and objectives of the case study so that students know what they will learn and how you expect them to demonstrate their learning. Next, remind students about their knowledge of speciation and extinction processes and the population as the fundamental unit of evolution. Students should be familiar with the difficulty of delimiting species given the conflict between speciation as a continuum and species designations as fixed categories (a difficulty reflected in the multitude of current species concepts). To contrast with what they already know, introduce the idea that the fundamental unit of conservation is the species. One way to do this is to deconstruct the language of the Endangered Species Act.

The Endangered Species Act is the centerpiece of conservation legislation in the United States. Enacted by President Nixon in 1973, it was designed to protect critically imperiled species from extinction as a consequence of human actions. The teaching presentation includes slides that interpret the letter of the law and highlight the focus of the legislation on *species* conservation. Students are not expected to read the legislation itself; the teaching presentation and the student worksheet should be informative enough that they have a basic understanding of the legislation.

At this point, pose the fundamental question of the Part I of the student worksheet: under the guidance of the Endangered Species Act, how should we conserve species as a product of evolutionary processes? Introduce the cutthroat trout species complex and the history of cutthroat trout in Colorado. The slides emphasize their diversity of form and distribution and their history of movement beyond isolated drainages by humans for subsistence, recreation, and conservation purposes with a focus on the greenback cutthroat trout. This introduction can be tailored to how much information you want your students to have before beginning the activity.

After introducing cutthroat trout and the Endangered Species Act, review the questions they should answer and how much time you will give them. Ask them to work in self-assembled groups of 2-4 students and make sure that each group has enough worksheets and figures. One worksheet can be submitted per group but students may find it easier if each person has their own worksheet to read and jot down notes.

Interruption I

While students are working on Part I, the instructor and any assistants should circulate through the classroom. Ask students to explain their answers and probe for any misunderstandings or confusion about the evidence. Supporting File 5 provides a rubric for responses to the student worksheet and includes common misconceptions and prompts to expand student thinking. Point students towards identifying uncertainty or error in the different types of evidence (e.g. overlapping counts in the morphological characters, branch lengths and lack of resolution in the phylogeny, hybrids in the STRUCTURE plot). After five to ten minutes of work time or until you notice a critical mass of confusion, redirect the students' attention back to the front of the class and spend two to three minutes describing how to interpret STRUCTURE plots. Most students will be unfamiliar with this type of analysis and figure; slides are provided in the teaching presentation to help describe how to read these types of figures. STRUCTURE analysis is a method to assign individuals to groups based on genotypes. It can be used to ask two types of questions: 1) given a certain number of groups, what is the probability that any individual belongs to a particular group, and 2) given a set of individuals, what is the most likely number of groups into which they can be assigned. The results of a STRUCTURE analysis can be used to infer population Structure (differentiation) and hybridization (gene flow), among other processes. After explaining STRUCTURE plots, ask the students to continue working to complete Part I of the student worksheet.

Introduce New Information

Circulate through the classroom to make sure that the majority of students have completed Part I. At this point, solicit groups to report their answers to each question. For question IA, emphasize how the different figures led to different answers; highlight common thought patterns by asking groups to raise their hands in response to which figures they found most informative and ask a few groups to share their reasoning. For question IB, ask a single group to describe the pattern, then emphasize alternative explanations, asking multiple groups to share their interpretations. For question IC, ask multiple groups to share their proposals and ask them why think their particular proposal will best address the question. We used responses from groups that proposed using historical specimens as a bridge to introduce Part II of the worksheet.

The second part of the case study asks, "what if we were wrong about the greenback cutthroat trout?" Through Part I, students should see that there is a discrepancy between where the so-called greenback cutthroat trout is found in the landscape (on both sides of the continental divide) and the historical range and description of the greenback (native to the South Platte and Arkansas River drainages on the eastern slope of the Continental Divide). Describe to the students the consternation caused by the publication of (16) as evidenced by the New York Times article, "After a Possible Oops, a Trout Rescue Project Regroups" (Oct. 13, 2007). After nearly half a century of conservation efforts to recover a species presumed extinct, the research suggested that conservation efforts had been misdirected at recovering and reintroducing a lineage that was non-native. Describe the research that addressed the confusion about the historic distributions of trout in Colorado by sequencing DNA from museum specimens of cutthroat trout collected prior to major stocking efforts (17). Ask students to evaluate the evidence used by (17) in question IIA.

Interruption II

While students are working on question IIA, circulate through the classroom and assess understanding of the figures. After two to five minutes, redirect the students' attention to the front of the class to give them more information on interpreting haplotype networks. This is another type of figure with which most students will be unfamiliar. You may need to remind students that a haplotype is a group of DNA bases or genes within an organism that are inherited together from a single parent (in contrast to the genotype, which is the set of genes inherited from both parents). A haplotype can come from a mitochondrial DNA sequence, which is maternally inherited; Y-chromosome DNA sequence, which is paternally inherited; or even from nuclear DNA sequence that only considers one chromosome and not it's homologous sister chromosome (students may find this reference helpful: http://www.nature.com/scitable/definition/haplotype-haplotypes-142). In this activity, students are considering only the first kind of data, mitochondrial sequences.

A haplotype network describes the relationship among such sequences; in such a network, each node is an actual or hypothesized haplotype and the vertices are the genetic distance between the haplotypes. You may find it helpful to watch a video (e.g. https://www.youtube.com/watch?v=SQ3JFaXJNpY) explaining the topic. After addressing any questions students may have about the interpretation of haplotype networks and the other figures for question IIA, ask them to complete question IIA and remind them to complete question IIB individually outside of class (or in class if time permits).

Conclude Lesson

To conclude the lesson, review student responses to Part II by asking groups to report their answers. Contrast their responses to question IIA with their responses to question IA, again emphasizing the uncertainty in the figures (e.g. branch lengths and polytomies [a node or branching point in a phylogeny that is not dichotomous and makes a comb or broomstick pattern] in the phylogeny, relationships among haplotypes in the network) and differences in conclusions among the different types of evidence. Ask for individual students proposals for management of the remaining population of greenback cutthroat trout, highlighting the nuances of balancing what they know about evolutionary biology and what they concluded based on the evidence with what they learned about the Endangered Species Act. Many students will propose some form of genetic rescue, the reintroduction of more distantly related individuals to increase genetic diversity and evolutionary fitness. Use student proposals for genetic rescue as a bridge to describe current research on the greenback cutthroat trout. Current research includes experimental crosses between the remnant greenback cutthroat trout population and another subspecies of cutthroat trout; the offspring from these crosses show a substantial recovery of fitness, evidence for genetic rescue of an inbred population. This research can be connected to other genetic rescue examples, such as the Florida panther (20), Swedish adder (21), and greater prairie chicken (22), among others. A strong ending note is that their proposals and the current research is timely because the state and federal agencies have already begun reintroduction efforts into the South Platte River drainage, the native range of the true greenback cutthroat trout.

<u>After Class Meeting</u>

Student Activities

Students should complete a brief post-assessment that to gauge changes in understanding of interpreting figures, knowledge of the Endangered Species Act, and opinions about the intersection of evolution and conservation.

Instructor Activities

Collect and compile student responses to the worksheet and pre- and post-assessments. Assign participation credit for completing the assessments and worksheet. Supporting File 1 provides a rubric for the pre- and post-assessments. Communicate with students if you discover any persistent misconceptions in the post-assessments.

TEACHING DISCUSSION

The goal of this activity is two-fold: (1) to encourage students to make connections between science curriculum in an evolution classroom and the challenges of conservation of endangered species; and (2) to provide students opportunities to confront uncertainty in real data sets and the possibility that different data sets will lead to different inferences about the same phenomenon. Both goals help prepare students for further academic achievement and to be engaged, science-literate citizens who can balance multiple viewpoints.

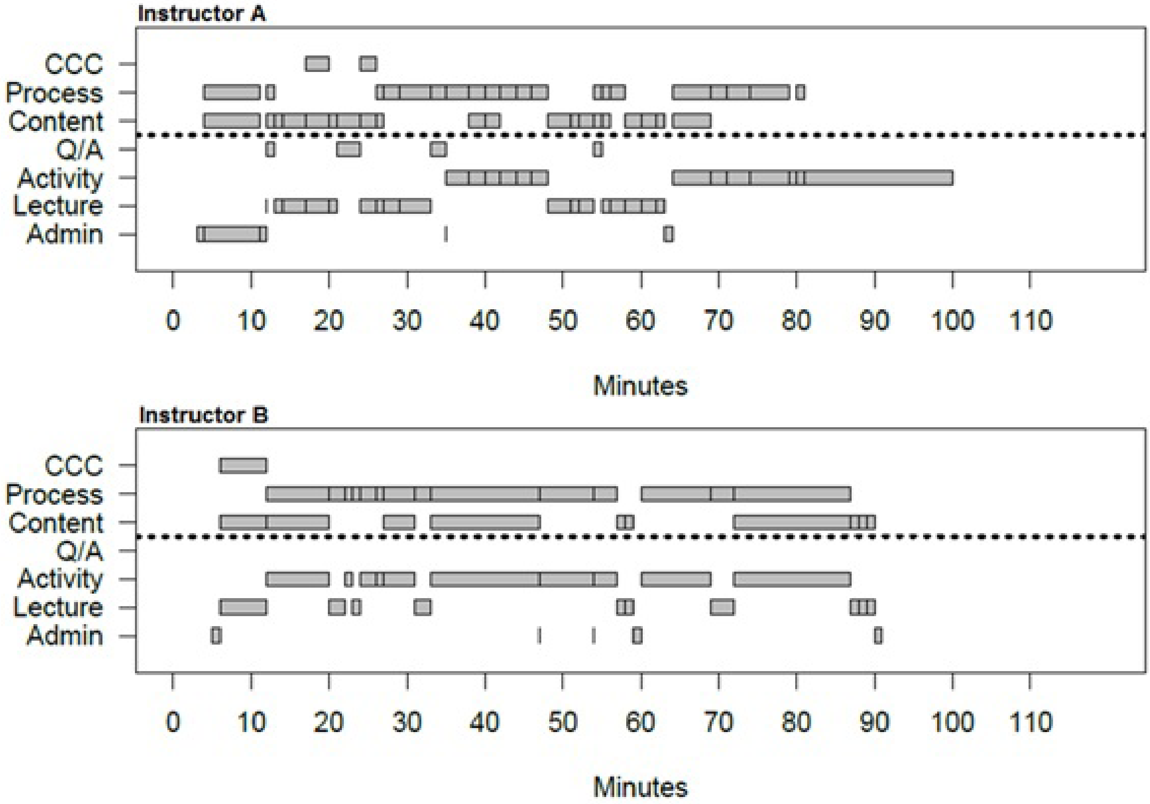

Figure 2. Instructor Time Budgets Observation of two different “naïve” instructors. We recorded time spent on cross-cutting concepts (CCC), process skills, and content, and on discussion, student activity, instructor lecture, and administrative tasks over the course of a single class period (fall 2015, 110 minutes, 19 students).

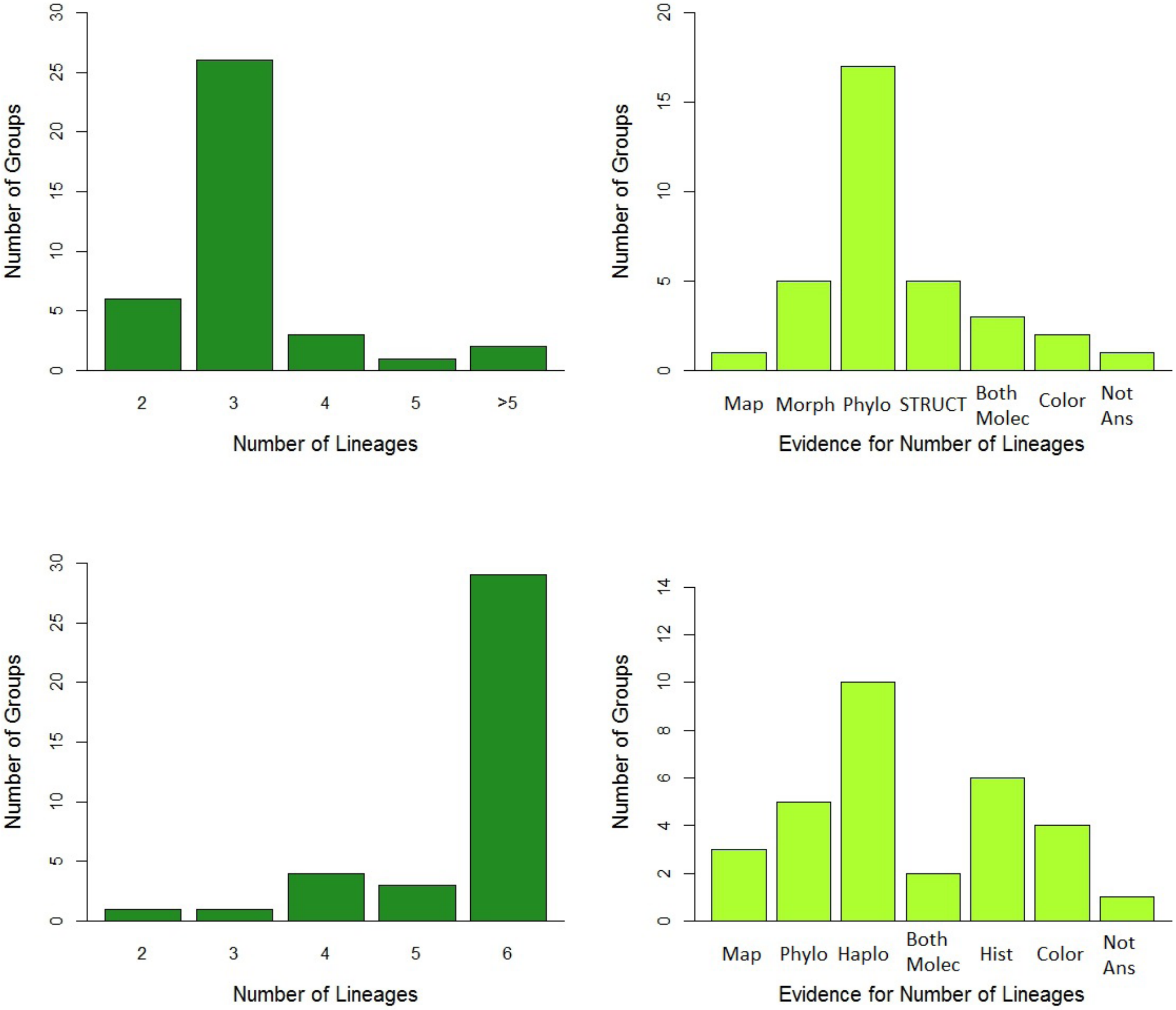

Figure 3. Student Responses to Worksheet Questions IA & IB. top left Student determination of the number of lineages present in Colorado based on data collected prior to 2007. Top right Types of evidence indicated by the students as being convincing evidence for their conclusions. Morpho = morphological data, Phylo = phylogeny, STRUC = STRUCTURE plot, Both Molec = both forms of molecular genetic data, Color = color scheme of figures, Not Ans = not answered. Bottom left Student determination of the number lineages present in Colorado based on data collected after 2007. Bottom right Types of evidence indicated as being convincing evidence for their conclusions. Haplo = haplotype network, Hist = historical stocking data. Total number of groups = 38 (all three occasions combined).

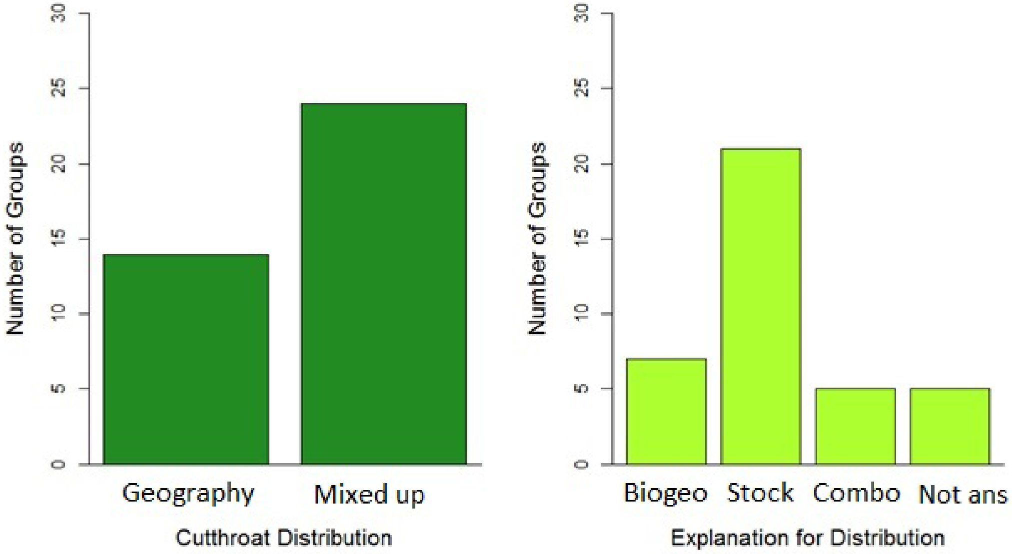

Figure 4. Student Responses to Worksheet Question IIA. Left Student descriptions of the distribution of cutthroat trout lineages in Colorado. Responses could be classified as either purely geographic, with each lineage confined to a single drainage, or mixed up, with lineages confined mostly to a single drainage but many populations out of place across the Continental Divide. Right Student explanations for the distribution described in the left figure. Biogeo = Biogeography, Stock = stocking, Combo = combination of biogeography and stocking, Not ans = not

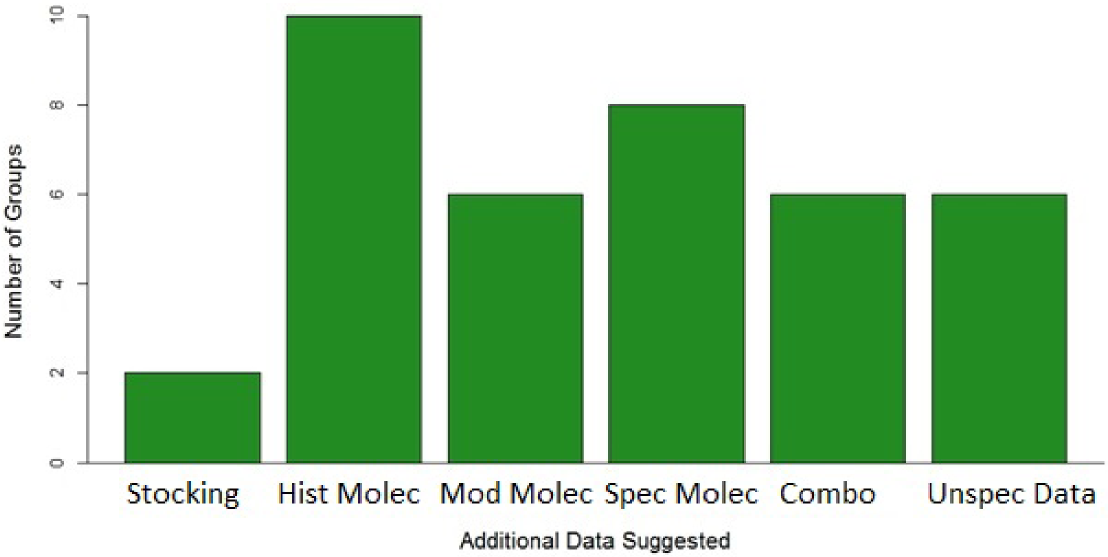

Figure 5. Student Responses to Worksheet Question IIIA. Student proposals for further research to determine the number and identity of cutthroat lineages native to Colorado prior to stocking. Hist Molec = molecular genetic data from historical samples, Mod Molec = molecular genetic data from modern samples, Spec Molec = specific molecular genetic technique or data, Combo = combination of data, Unspec = unspecified data. Total number of groups = 38 (all three occasions combined).

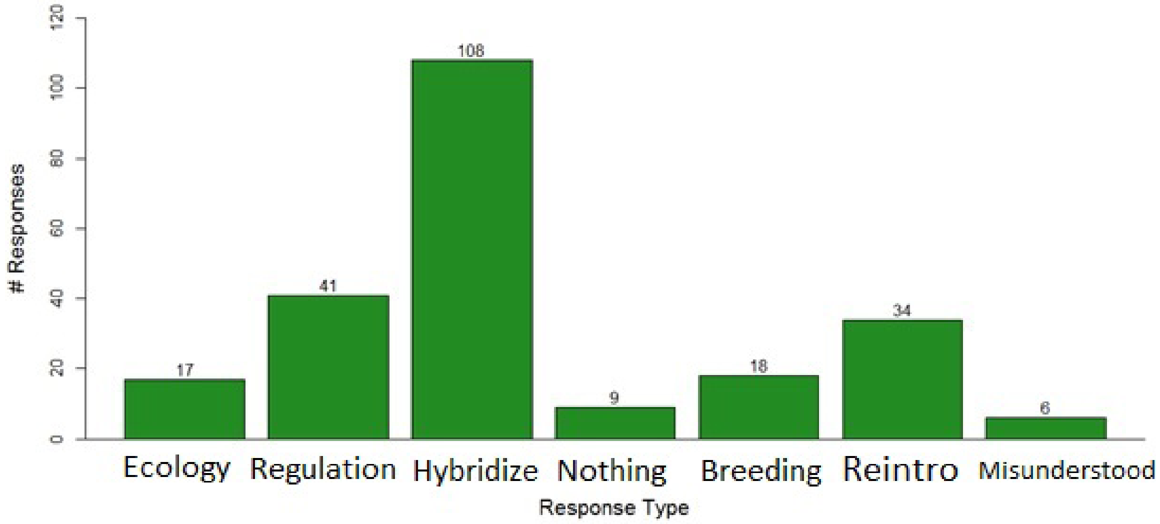

Figure 6. Student Responses to Question IIB. Student proposals for management action to conserve the remaining greenback cutthroat trout. Ecology = study or protections based on ecology, Regulation = federal protections, regulated fishing or access, Hybridize = outcrossing or genetic rescue, Nothing = take no action, Breeding = controlled breeding program, Reintro = reintroduce into native range, Misunderstood = misunderstood question.

Effectiveness

To quantify the effectiveness of the case study, we implemented pre- and post-assessments the third time we taught the material (spring semester of the large lecture section). Out of 101 students, 66 completed both the pre- and post-assessments (questions and rubric available in Supporting File 1). Questions 1 and 2 were about reading and interpreting phylogenies. Question 3 addressed student knowledge of the Endangered Species Act and the status of hybrid organisms. Question 4 addressed student familiarity with STRUCTURE plot. Question 5 asked whether the effect of hybridization was positive, negative, or context-dependent. We calculated normalized learning gains for each question but found that that the questions were not perfectly isomorphic and so do not reflect changes in student thinking accurately. Instructors may wish to use Supporting File 1 as a question bank from which to draw and use the exact same questions for pre- and post-assessment. Overall, the assessment questions provided insight into student thinking (discussed below) and allowed us to improve the lesson based on student misconceptions (Supporting File 5 discusses misconceptions).

To further improve the lesson, we asked two other instructors for the Evolution course who were not knowledgeable about the cutthroat trout system to implement the lesson in their classrooms (110-minutes, 19 students each). This experiment gave us an opportunity to observe how other instructors taught the material and identify any areas that were confusing to instructors and students. Figure 2 (on page 6) describes the time budget of the two different instructors and shows how instructors may adjust their balance of lecture and student activity. Instructor B gave more frequent, shorter interruptions while Instructor A started their instruction with a long lecture and gave longer lectures over the duration of the session.

Overall, students appeared to be highly engaged in the lesson, and with some guidance from the instructors, undergraduate learning assistants, and graduate teaching assistants, were able to appreciate the complexity and uncertainty inherent in the data. In a small classroom, guiding small groups will be manageable for a single instructor but in the larger classroom additional support was helpful for deepening thinking and addressing misconceptions. Supporting File 5 is a rubric for the student worksheet and gives suggestions for questions to prompt deeper thinking and understanding of the figures. Instructors interested in student perceptions of their own learning and opinions about conservation may consider developing a short Student Assessment of Learning Gains tool (www.salgsite.org).

Student Responses

Number of Lineages & Weighing Evidence

Most students arrived at the conclusions proposed by the authors of Metcalf et al. 2007, 2014 (Figure 3, on page 7): namely, that prior to the use of museum specimens, genetic evidence supported the conclusion that three cutthroat trout lineages were native to Colorado. The use of museum specimens then provided evidence that at least six lineages were native to Colorado. Students relied mainly on molecular evidence (choosing the phylogeny or haplotype network as the most convincing) (Figure 3), which may reflect their familiarity with reading phylogenies and their lack of familiarity with other kinds of evidence, such as STRUCTURE plots or maps. Student responses to verbal questioning about inconsistencies or uncertainty in the different forms of evidence were generally more sophisticated than the responses recorded in the worksheet. An alternative strategy would be to ask the students to report the general conclusions and major uncertainties for each figure, rather than asking them to draw a single conclusion. Another suggestion would be to assign a single figure per small group which describes the conclusions and uncertainties for that figure, then small groups with different figures explain their figures to one another and come to a consensus. In that way, groups are only responsible for the density of information in a single figure, rather than attempting to interpret multiple complex figures.

Sources of Uncertainty & Proposing Additional Research

Most students correctly identified that the distribution of cutthroat trout lineages reflected both geography with the Continental Divide as a barrier and human action as stocking moved lineages across the Continental Divide (Figure 3). The number of students describing the distribution based on geography (Figure 3 left) suggests that instructors should focus on questioning how cutthroat trout may have crossed a major barrier to movement (the Divide stands above 14,000 ft in some places in Colorado) when the molecular evidence shows that they are clearly and deeply diverged from other lineages. Students should be prompted to carefully inspect the maps in Student Figure 5, on page 8. Additionally, the number of students attributing the distribution solely to stocking (Figure 3 right) suggests that instructors should remind students to consider a more complicated explanation that accounts for both deep evolutionary time (divergence in isolated drainages) and more recent human actions (stocking).

Students honed in on using molecular genetic evidence to better understand how many lineages were native to Colorado (Figure 4, on page 8). Students frequently suggested using a particular molecular marker or gene to gain deeper insight. With probing questions intended to motivate students to think more carefully about what their research would actually yield, many students suggested using molecular genetic evidence from historic samples collected prior to stocking. This suggestion provided a perfect segue into the next portion of the lesson, which describes research by Metcalf et al. (2014) that used museum specimens of cutthroat trout to uncover the number of lineages native to Colorado prior to stocking.

Proposing Conservation Actions

The depth and detail of some student responses to the worksheet questions, especially the final question asking them to make management recommendations, showed great enthusiasm for the topic and an attempt to reconcile their knowledge from the course with the constraints of legislation and wildlife management. For instance, one student wrote a formal letter to Colorado Parks & Wildlife:

I believe we need to introduce regulations to help protect this population of native Cutthroat Trout. First, I believe fish stocking should be banned in the Arkansas drainage. This will greatly decline competition between non-native and native trout, and will allow native populations to grow again to stable numbers. Second, I believe we should ban all fishing within the three-mile stretch of river where this population is located. This will decrease the amount of predation the fish of this population are exposed to, since humans are a main predator of these fish. Third, I believe catch-and-release fishing should be required for all Cutthroat Trout. It is often hard to distinguish between subspecies of Cutthroats, and this will allow all populations to have a better chance of survival.

This response is an example of the call by many students for increased or altered regulation of the management of the greenback cutthroat trout (Figure 5, on page 8). Many students wrote about the need to gather additional ecological data and alter the language of the Endangered Species Act to allow for more flexibility in considering hybrids. Most students called for some form of hybridization or genetic rescue, wherein the inbred greenback cutthroat trout would be crossed with a related trout to increase genetic diversity and fitness (Figure 6, on page 9):

Since the genetic diversity of this cutthroat trout population is so small, it is in danger of extinction due to negative health effects of inbreeding like deformities and reduced survival rate. The population needs an influx of new genotypes to mate with in order to preserve the lineage. However, it would be ecologically unsound to introduce non-native trout species to the area because they could negatively effect [sic] the ecosystem since they aren't native. Thus, it seems most logical to remove gametes from the population and induce artificial fertilization back in the lab with the gametes of some closely related trout.

This response reflects high level thinking about the consequences of genetic rescue.

The suggestion by students to attempt genetic rescue is a good opportunity to describe current research and management efforts as a conclusion to the lesson. Researchers crossed gametes from the inbred population with another subspecies; the resulting hybrids showed a significant recovery of fitness compared to inbred crosses (K. Rogers unpublished data). This research is timely as the state and federal agencies began reintroducing greenback cutthroat into their native range in 2014, using inbred hatchery stock. Further research is using controlled crosses in the hatchery to alleviate the effects of inbreeding so that future reintroductions use fitter, more genetically diverse stock.

While cutthroat trout are an obvious choice for a case study to engage students in Colorado, the plight of these fish is representative of many threatened species around the world. In addition to using this case study as a microcosm of the intersection of evolution (speciation is a continuum, hybridization happens), scientific thinking (weighing evidence, accounting for uncertainty), and conservation legislation (the Endangered Species Act), it could be adapted for other organisms that face the genetic consequences of small population size and that may be more charismatic or locally exciting (e.g. Florida panthers or Isle Royale wolves). Students readily engage with conservation scenarios and have many ideas for research and management that are novel or reflected in the literature, both of which can be validating for students who may have limited experience reading primary literature or applying scientific information outside of the classroom.

SUPPORTING MATERIALS

- S1. Cutthroat Case Study Pre-Post Assessment Rubric (23-27)
- S2. Cutthroat Case Study Student Worksheet
- S3. Cutthroat Case Study Lecture Slides
- S4. Cutthroat Case Study Student Figures
- S5. Cutthroat Case Study Worksheet Rubric
- S6. Cutthroat Case Study Worksheet Rubric

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Table 1. Cutthroat Trout Teaching Timeline

Activity	Description	Estimated Time (min)	Learning Goals & Objectives	Notes
Preparation for class				
Instructor preparation	1. Skim (16,17) 2. Review lecture and student worksheet to align with your style and learning goals 3. Make one black and white copy of the 7 figures (Supporting files) for each group of 2-4 students 4. Make one color copy of the 7 figures for every 2 or 3 group of 2-4 students 5. Make one copy of the student worksheet for each student	60-120		Figures = Supporting file S4 Student worksheet = Supporting file S2 Presentation = Supporting file S3
Student preparation	Complete pre-assessment (can be set up through course management software, clickers, or hardcopy)	5-10	• Understand how the Endangered Species Act treats evolutionary processes • Connect evolutionary concepts to wildlife management practices	Questions = Supporting file S1
Class meeting I (50 minutes)				
Introduce the activity	1. Review the learning goals and objectives 2. Introduce the system: describe cutthroat trout and where they are found 3. Connect evolution to conservation 4. Introduce the Endangered Species Act	1. 2-3 2. 2-3 3. 1 4. 3-5	• Understand how the Endangered Species Act treats evolutionary processes • Connect evolutionary concepts to wildlife management practices	Presentation = Supporting file S3
Student work time	Distribute worksheet and start working through questions IA-C	5-10	• Able to interpret figures • How different kinds of data lead to different conclusions • Identify sources of uncertainty and disagreement	Circulate through groups
Interruption I	Explain STRUCTURE plots	2-3	• Able to interpret figures • Identify sources of uncertainty and disagreement	Interrupt after asking several groups whether they understand Figure 2
Student work time	Complete questions IA-C	5-10	• Able to interpret figures • Identify sources of uncertainty and disagreement • Propose research to remedy uncertainty	
Expand the activity	1. Review student responses to questions IA, IB, IC 2. Introduce Part II of the lesson	5-10	• Understand how the Endangered Species Act treats evolutionary processes • Connect evolutionary concepts to wildlife management practices	Good time to break if meeting time is concluded (before introducing Part II)
Student work time	Start working through questions IIA	5	• Able to interpret figures • How different kinds of data lead to different conclusions • Identify sources of uncertainty and disagreement	Circulate through groups

Activity	Description	Estimated Time (min)	Learning Goals & Objectives	Notes
Interruption II	Explain haplotype networks	2-3	• Able to interpret figures • Identify sources of uncertainty and disagreement	Interrupt after asking several groups whether they understand Figure 6
Student work time	Complete question IIA, brainstorm response to IIB	5	• Able to interpret figures • How different kinds of data lead to different conclusions • Identify sources of uncertainty and disagreement	
Conclude the activity	1. Review student responses to questions IIA 2. Remind students to complete IIB outside of class	5-10	• How different kinds of data lead to different conclusions • Identify sources of uncertainty and disagreement	Emphasize the contrast between their answers for IA and IIA, the number of lineages in Colorado based on different kinds of evidence
After class meeting I				
Student completion	Answer final question in form of short answer (can be submitted electronically to course management software)	5-10	• Understand how the Endangered Species Act treats evolutionary processes • Connect evolutionary concepts to wildlife management practices	
Class meeting II (10-20 minutes)				
Wrap up the activity	1. Review student responses to IIB 2. Present current research 3. Remind students to complete post-assessment	1. 5-10 2. 5-10 3. 1	• Connect evolutionary concepts to wildlife management practices	
After class meeting II				
Student completion	Complete post-assessment (through course management software)	5		
Instructor completion	Review student responses, assign credit for participation	20-30		Pre/post-assessments can be compared to examine learning