NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Combatting the Southern Pine Beetle:

RNAi, Science Policy, and Working with Indigenous Communities

by

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Introduction

Nicole sat down with her grandpa after class one afternoon, turning on the television to see the latest news in their hometown in rural Virginia. Another fire had started in a nearby forest fueled by the dry, girdled logs of the pine trees. "It's a shame," he said to Nicole, "our lands are being destroyed. When our ancestors lived here, the trees were plentiful and provided us with so much. Now, they sit here and die, fueling the next great forest fire and burning down our beautiful landscape."

Nicole's grandpa went on, explaining the importance of the pine tree to their family.

"Pine trees hold a sacred place in our culture. Generations of Indigenous communities learned the art of canoe-building from their elders, passing down the knowledge like a treasured heirloom. To Indigenous people, the tree was used in every aspect of their lives. The tree's bark was carefully harvested, and the trunk was transformed into sturdy vessels for fishing and traveling. The tree's needles and sap were key medicines, helping to heal wounds and ease pain. The pine needles were even made into tea to soothe coughs during the winter months.

"As travel and trade increased, so did the pine tree and its foe, the southern pine beetle. Over the last fifty years, the beetle has traveled its way from Mississippi and Alabama up to New England, taking out forests of pine, hemlock, and spruce species. Our communities have been severely impacted by the loss of so many trees, and the future of the pine tree is in danger."

"The southern pine beetle is the reason for all these fires and forest losses? This is just anoher example of how invasive species are overtaking our homelands!" said Nicole.

"It's a shame there isn't an easy way to control invasive species these days. Insecticides and biological control can only go so far, but we need to start looking at how to manage these targeted populations without killing all the surrounding nature," her grandpa responded.

Nicole, a biologist at the nearby university, responded to her grandpa, "You know, there's a new technology that uses a host's immune system against itself. Maybe we can find a way to target the southern pine beetle population without affecting the ecosystem. For example, what if the insects can lose the ability to grow large wings? If it can't fly to a new location, it can't reproduce and keep eating the trees."

At that moment, it clicked. Nicole knew what she wanted to do for her next research project. She wanted to design a way to combat the southern pine beetle using this new method, saving the tree population for her community.

Figure 1. Southern pine beetle (*Dendroctonus frontalis*). *Credit:* Erich G. Vallery, USDA Forest Service - SRS-4552, Bugwood.org, CC BY-NC 3.0 US.

Preparation for Part I

Read the following article and then answer the questions below covering the basics of RNA interference (RNAi):

- Christiaens, O., S. Whyard, A.M. Vélez, & G. Smagghe. (2020). Double-stranded RNA technology to control insect pests: current status and challenges. *Frontiers in Plant Science* 11, 451. <https://doi.org/10.3389/fpls.2020.00451>

Questions

1. RNAi targets the _____ found within the target cell, preventing _____ synthesis.

2. Compare and contrast reasons to use RNAi over CRISPR or T-DNA insertions.

3. Define the terms below:

   siRNA vs. miRNA

   DICER

   RISC

   AGO

4. Name one organismal and one environmental factor that affects the stability of dsRNA.

5. Based upon information in the article, briefly explain the difference between HIGS, SIGS, and VIGS in a plant-pathogen interaction. Include a definition, example, advantage, and disadvantage of each technique. Which one do you think we should use for the southern pine beetle, and why?

Part I – Setting up the Experiment

You and Nicole have been hired by the USDA and a local tribal conservation office to develop a control strategy for the southern pine beetle (SPB) in New England using the insect's endogenous RNAi machinery. Newer studies are showing that dsRNA treatment on trees is beneficial for controlling insect populations, and now it is your turn to test that hypothesis on the SPB.

Questions

1. Compare the RNAi methods you chose for Question 5 of the "Preparation for Part I" assignment. Explain why each method will or will not work in controlling the SPB population.

2. Name three possible gene targets for shRNA that will help in managing the SPB population (e.g., gene regulating wing size). Explain why you chose each.

3. Design the Ti plasmid carrying an RNAi construct built for short-hairpin RNA. Be sure to label and define the following: (1) origin of replication, (2) antibiotic resistance, (3) promoter, and a short hairpin RNA (shRNA) template including (4) the sense sequence, (5) stem loop, and (6) antisense sequence.

4. After constructing the recombinant plasmid, name two experimental methods that could be used to transform the bacteria with your plasmid.

5. Which organism is the recipient of the RNAi construct?

6. Describe what happens to the RNAi construct once transformed into the bacteria and how you end up with short-hairpin RNA for treatment.

After some hard work in the lab, you've successfully created your constructs targeting two genes for each of your answers in Question 2. You screen the vectors for a physiological change in the bug's anatomy, metabolism, or behavior.

Questions

7. In screening your RNAi vectors, what would be an effective physiological change in the SPB? Why?

8. Would you want the gene of interest to affect the current generation of SPB or future generations? Explain.

9. Identify the delivery vehicle and the recipient of the purified short-hairpin RNA.

Preparation for Part II

Exciting news! You and Nicole have identified a promising candidate gene for addressing the SPB spread through RNAi technology. This gene codes for the protein DIGEST1, an enzyme thought to be responsible for feeding and digestion. Your next task involves transitioning this breakthrough from the laboratory to practical applications in the real world.

As a first step, you are designing a greenhouse trial to test the sprayed dsRNA on a small population of SPBs. You will be using two types of trees (pine and hemlock), your dsRNA spray mix targeting DIGEST1, and two types of beetles: the invasive southern pine beetle and the native mountain pine beetle. The mountain pine beetle, unlike the SPB, is native to the region and only typically targets trees that are already under severe stress.

Questions

1. Write a testable hypothesis for your RNAi insect control experiment.

2. Sketch out a design of your experiment. Be sure to include controls for each type of treatment, and think about the different variables (insect, vector, tree, etc.).

3. Why is it important that we are treating the mountain pine beetle alongside our targeted southern pine beetle?

4. If the mountain pine beetle population dies during our dsRNA trials, what part of our experiment must we alter? Explain.

Assume your greenhouse trials succeed, and your RNAi-based insect control is approved for use in the northeastern regions of the United States. Before spraying an entire forest, you must get approval from the federal government.

In 2021, the White House Office of Science and Technology Policy and the White House Council on Environmental Quality released a memorandum committing to elevating Indigenous traditional ecological knowledge (ITEK) in federal scientific and policy processes. Read the memorandum (attached below):

- Executive Office of the President. (2021, November 15). Memorandum for the heads of departments and agencies: Indigenous traditional ecological knowledge and federal decision making.

The memorandum requires that you consider ITEK in your implementation of the RNAi-based insect control strategy, as the Eastern Band of Cherokee Nation covers a large part of the forest you are interested in treating with your RNAi design. You are fortunate enough that Nicole has brought in two local tribal officers to help facilitate consultations with the tribal nations most affected by the decline of the pine trees in the region.

Questions

5. According to the memorandum, what is the definition of ITEK?

6. How do ITEK and Western scientific knowledge work together in this context of conservation? Are there specific areas where ITEK provides unique insights or perspectives that you may not have thought of?

EXECUTIVE OFFICE OF THE PRESIDENT

OFFICE OF SCIENCE AND TECHNOLOGY POLICY

COUNCIL ON ENVIRONMENTAL QUALITY

WASHINGTON, D.C.

November 15, 2021

MEMORANDUM FOR THE HEADS OF DEPARTMENTS AND AGENCIES

FROM: Eric S. Lander
President's Science Advisor and Director,
Office of Science and Technology Policy

Brenda Mallory
Chair, Council on Environmental Quality

SUBJECT: Indigenous Traditional Ecological Knowledge and Federal Decision Making

Background

President Biden is committed to strengthening the relationship between the Federal Government and Tribal Nations and to advancing equity for Indigenous people, including Native Americans, Alaska Natives, Native Hawaiians, and Indigenous peoples of the U.S. territories.[1] These commitments include ensuring that Federal agencies conduct regular, meaningful, and robust consultation with Tribal officials in the development of federal research, policies, and decisions, especially decisions that may affect Tribal Nations and the people they represent.

Consistent with the Administration's additional commitment to scientific integrity and knowledge- and evidence-based policymaking,[2] the White House Office of Science and Technology Policy (OSTP) and the White House Council on Environmental Quality (CEQ) issue this memorandum to recognize Indigenous Traditional Ecological Knowledge (ITEK)—a form of Indigenous Knowledge[3]—as one of the many important bodies of knowledge that contributes to the scientific, technical, social, and economic advancements of the United States and to our collective understanding of the natural world.

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[1] Memorandum on Tribal Consultation and Strengthening Nation-to-Nation Relationships, 86 Fed. Reg. 7,491 (Jan. 26, 2021); Executive Order 13,985: Advancing Racial Equity and Support for Underserved Communities Through the Federal Government, 86 Fed. Reg. 7,009 (Jan. 20, 2021); Executive Order 14,031: Advancing Equity, Justice, and Opportunity for Asian Americans, Native Hawaiians, and Pacific Islanders, 86 Fed. Reg. 29,675 (May 28, 2021).

[2] Memorandum on Restoring Trust in Government Through Scientific Integrity and Evidence-Based Policymaking (Jan. 27, 2021), https://www.whitehouse.gov/briefing-room/presidential-actions/2021/01/27/memorandum-on-restoring-trust-in-government-through-scientific-integrity-and-evidence-based-policymaking/.

[3] A variety of terms and definitions are used by knowledge holders, Indigenous people, Tribal organizations, and government bodies to refer to this body of knowledge or related concepts, including "Indigenous Knowledge," "Indigenous Knowledges," and "Traditional and Indigenous Knowledge." This memorandum will use "Indigenous Traditional Ecological Knowledge," or "ITEK," to refer to Indigenous Knowledge that pertains to ecology and the environment, although OSTP and CEQ intend to seek input on the appropriate terms to use in this context.

ITEK is a body of observations, oral and written knowledge, practices, and beliefs that promote environmental sustainability and the responsible stewardship of natural resources through relationships between humans and environmental systems. It is applied to phenomena across biological, physical, cultural and spiritual systems. ITEK has evolved over millennia, continues to evolve, and includes insights based on evidence acquired through direct contact with the environment and long-term experiences, as well as extensive observations, lessons, and skills passed from generation to generation. ITEK is owned by Indigenous people—including, but not limited to, Tribal Nations, Native Americans, Alaska Natives, and Native Hawaiians.[4]

The Federal Government has previously received requests to develop guidance for Federal agencies on how to partner with Tribal Nations and Native organizations regarding the application of ITEK.[5] The Administration recognizes that the Federal Government should engage with ITEK only through relationships with Tribal Nations and Native communities and in a manner that respects the rights of knowledge holders to control access to their knowledge, to grant or withhold permission, and to dictate the terms of its application. It further recognizes that, should Tribal Nations and Native communities decide to share ITEK and otherwise collaborate with the Federal Government, the Federal Government should ensure that the application of that knowledge and complementary collaborative efforts benefit Tribal Nations, Native communities, the United States, and our planet.

With these principles in mind, OSTP and CEQ are initiating a process to develop government-wide guidance for Federal agencies on ITEK, with Tribal consultation and drawing on the important work that has already occurred at a number of agencies and within Tribal Nations and Native communities. This memorandum is the first step in that process, which will be shaped by the input of Tribal Nations, ITEK holders and practitioners, Federal agency experts, and the public.

Indigenous Traditional Ecological Knowledge and Federal Decision Making

Where appropriate, ITEK can and should inform Federal decision making along with scientific inquiry. Indeed, the Fourth National Climate Assessment recognized and incorporated ITEK as an important information source for improving the understanding of climate change and environmental sustainability over time, and for developing comprehensive climate adaptation and natural resource management strategies.[6] As the examples provided at the end of this memorandum show, Tribal Nations and Native communities have already worked effectively

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[4] U.S. Fish & Wildlife Service, *Traditional Ecological Knowledge Fact Sheet* (Feb. 2011), https://www.fws.gov/nativeamerican/pdf/tek-fact-sheet.pdf; Inuit Circumpolar Council, *Indigenous Knowledge*, https://www.inuitcircumpolar.com/icc-activities/environment-sustainable-development/indigenous-knowledge/.

[5] National Congress of American Indians, *Request for Federal Government to Develop Guidance on Recognizing Tribal Sovereign Jurisdiction over Traditional Knowledge* (2013), https://www.ncai.org/resources/resolutions/request-for-federal-government-to-develop-guidance-on-recognizing-tribal-sovereign-jurisdiction-over-traditional-knowledge.

[6] Fourth National Climate Assessment, *Tribes and Indigenous Peoples*, Volume II, Chapter 15 (2018), https://nca2018.globalchange.gov/downloads/NCA4_Ch15_Tribes-and-Indigenous-Peoples_Full.pdf.

with Federal agencies to incorporate ITEK into knowledge- and evidence-based Federal Government decision making, and such collaborations have been mutually beneficial.

The guidance that OSTP and CEQ plan to develop—with Federal agency collaboration, robust and meaningful Tribal consultation, and input from ITEK holders and practitioners and the public—will include best practices on how to collaborate with Tribal Nations and Native communities around ITEK application to achieve mutually beneficial outcomes, how to address Federal Government-wide challenges around ITEK such as navigating Federal laws and interagency processes, and how to appropriately respect the knowledge holders' rights to decline participation in efforts to collaborate. The guidance will be designed to complement, not supplant, existing agency guidance related to ITEK and will build on past efforts to recognize and incorporate ITEK into Federal scientific and policy decisions.

Establishment of Interagency Working Group

OSTP and CEQ will begin in 2021 by developing pathways for Tribal and Native community input and convening an interagency working group to inform the development of the guidance. This "Interagency Working Group on Indigenous Traditional Ecological Knowledge" will include representatives from agencies across the Federal Government. The purpose of the working group is to enhance interagency collaboration and coordination, draw on agency experience, and address significant issues as they arise. The Interagency Working Group on Indigenous Traditional Ecological Knowledge will prepare the guidance document for planned release in 2022.

OSTP and CEQ look forward to collaborating with Tribal Nations and Native communities to apply ITEK in a way that benefits people and the planet and facilitates a holistic understanding of the world.

APPENDIX: Supporting Examples of ITEK Application and Collaboration Between Native Communities and the Federal Government, Written With Native Partners

The Administration recognizes that, for generations, Federal policies have systematically sought to assimilate and displace Indigenous people and to eradicate Indigenous cultures. The below examples are intended not to erase this history, but to illustrate the kinds of mutually beneficial collaborations that are possible. These examples include input from and reflect the perspectives of the Native organizations, individuals, and agencies involved in these particular efforts.

- Coastal Indian Tribes, including the Cowlitz Indian Tribe, have fished and traded for eulachon in tributaries of the Columbia River since time immemorial. NOAA and the Cowlitz Indian Tribe—who initiated the project—applied Tribal oral histories to reconstruct historic distributions of the eulachon, a species of fish.[7] Those Cowlitz Tribal oral histories aided in identification of key spawning habitat, timing of eulachon runs, and run differences between tributaries and directly informed NOAA's decision to list a population segment as threatened under the Endangered Species Act.[8] The project facilitated joint efforts to identify and protect critical habitat, increase abundance of the species, and promote species recovery.[9]
- In Acadia National Park, the National Park Service is working with citizens of Wabanaki Tribes—the Aroostook Band of Micmacs, the Houlton Band of Maliseets, the Passamaquoddy Tribe at Sipayak, the Passamaquoddy Tribe at Indian Township, and the Penobscot Indian Nation—on shared governance and research on sweetgrass harvesting.[10] Wabanaki people have harvested sweetgrass for generations. Research in Acadia, guided by Indigenous methodologies, reinforces what Wabanaki people have always known: that harvesting sweetgrass through a Wabanaki philosophy enhances sweetgrass abundance. Wabanaki knowledge, and the gatherers who generate this knowledge, are leading NPS research and management strategies that will enable restoration of Wabanaki harvesting within Acadia National Park.[11]
- For Native Hawaiians, cultural heritage and the natural world are valued as one. At Papahānaumokuākea Marine National Monument in the Northwestern Hawaiian Islands, this ancestral, cultural, and natural significance are on an equal platform with all other

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[7] Nathan Reynolds, Marc Romano, *Traditional Ecological Knowledge: Reconstructing Historical Run Timing and Spawning Distribution of Eulachon through Tribal Oral History*, Journal of Northwest Anthropology (2013).

[8] National Marine Fisheries Service, *Recovery Plan for the Southern Distinct Population Segment of Eulachon*, (Sept. 2017), https://repository.library.noaa.gov/view/noaa/15989.

[9] NOAA Fisheries, National Ocean Service, *Guidance and Best Practices for Engaging and Incorporating Traditional Ecological Knowledge in Decision-Making* (May 2019), https://www.legislative.noaa.gov/docs/19-065933-Traditional-Knowledge-in-Decision-Making-Document-Signed.pdf.

[10] U.S. Forest Service Southern Research Station, *Traditional Ecological Knowledge Helps Researchers Understand the Effects of Plant Harvesting* (2018), https://srs.fs.usda.gov/research/2018-research-highlights/highlight.php?id=traditional-knowledge.

[11] Abbe Museum, *Wabanaki Sweetgrass Harvesting in Acadia National Park* (June 1, 2019), https://www.abbemuseum.org/blog/2018/6/21/a8ox8s8wxde6nenklfm77gayl60h87.

interests.[12] The monument is co-managed by the National Oceanic and Atmospheric Administration, U.S. Fish and Wildlife Service, the State of Hawai'i Office of Hawaiian Affairs, and the Hawai'i Department of Land and Natural Resources, and Native Hawaiians have consistently led the development and governance of the monument. Its management is based on Indigenous Hawaiian Knowledge and management practices, as expressed in the recent release of Mai Ka Pō Mai, a historic guidance document that will help federal and state agencies further integrate Native Hawaiian culture into all aspects of management.[13]

- The Inupiat, St. Lawrence Island Yupik, Central Yup'ik and Cup'ik peoples have lived in the Northern Bering Sea region for millennia interconnected with marine and coastal ecosystems. Tribes, regional Alaska native non-profit organizations, Elders and Traditional Knowledge holders from across the northern Bering Sea region worked with the Federal government over concerns about rapid climate change and the need for solutions that take a whole-of-government approach that build equity in decision-making for the Northern Bering Sea region. The Northern Bering Sea Climate Resilience Area, established by Executive Order 13,754 in 2016, and reinstated by President Biden in 2021, provides an example of Indigenous values informing policy and the potential for including Traditional Knowledge in decision-making.[14] It provides a model for bridging different value systems coming from Indigenous Knowledge and academic science through a framework that includes a federal task force and Bering Intergovernmental Tribal Advisory Council. The task force and advisory council are charged with conserving the region's ecosystem, including those natural resources that provide important food security to the people of the region. It also provides a pathway for Tribal voices that have been historically underserved in decision-making processes.

[12] Papahānaumokuākea Marine National Monument, *2020 State of the Monument Report* (2020), https://sanctuaries.noaa.gov/science/condition/pmnm/welcome.html

[13] Papahānaumokuākea Marine National Monument, *Integrating Native Hawaiian Culture into Management of Papahānaumokuākea* (June 21, 2021), https://www.papahanaumokuakea.gov/new-news/2021/06/21/maikapomai/.

[14] Executive Order 13,754: Northern Bering Sea Climate Resilience, 81 Fed. Reg. 90,669 (Dec. 9. 2016); Press Release: Biden-Harris Administration Brings Arctic Policy to the Forefront (Sept. 24, 2021), https://www.whitehouse.gov/ostp/news-updates/2021/09/24/biden-harris-administration-brings-arctic-policy-to-the-forefront-with-reactivated-steering-committee-new-slate-of-research-commissioners/.

Part II – The Importance of Scientific Communication

After meeting with the tribal officials and altering your plan of action, you must report on your findings from both the laboratory and greenhouse experiments to the USDA, fellow entomologists, and the Eastern Band of the Cherokee Nation.

For the first part of class, choose *one* of the following audiences and write your report so that it addresses their interests, keeping in mind the following:

- *Fellow entomologists:* include scientific details of RNAi technology, its mechanism, and how it targets SPBs. Discuss the benefits of RNAi and mention any potential challenges that entomologists might be concerned about.
- *United States Department of Agriculture (USDA):* emphasize the potential benefits of RNAi technology for forest management, its impact on curbing SPB infestations, and how it aligns with the USDA's goals and policies, including ITEK.
- *The Eastern Band of the Cherokee Nation:* Assuming that the tribal members are not trained in Western science, prepare a presentation to the tribe using terminology that can be understood by a non-scientific audience to explain what RNAi technology is and how it can potentially benefit the community by protecting forests from SPB infestations. Address any potential safety or environmental concerns and include information on your consideration of ITEK and your consultation with tribal officers.

Supporting Data for Your Report

The data below include three figures (Figures 1–3) showing results from the laboratory experiments, a table (Table 1) showing results from the greenhouse experiment, and a summary of your meeting with tribal leaders of the Eastern Band of the Cherokee Nation. Be sure to include this data in your report.

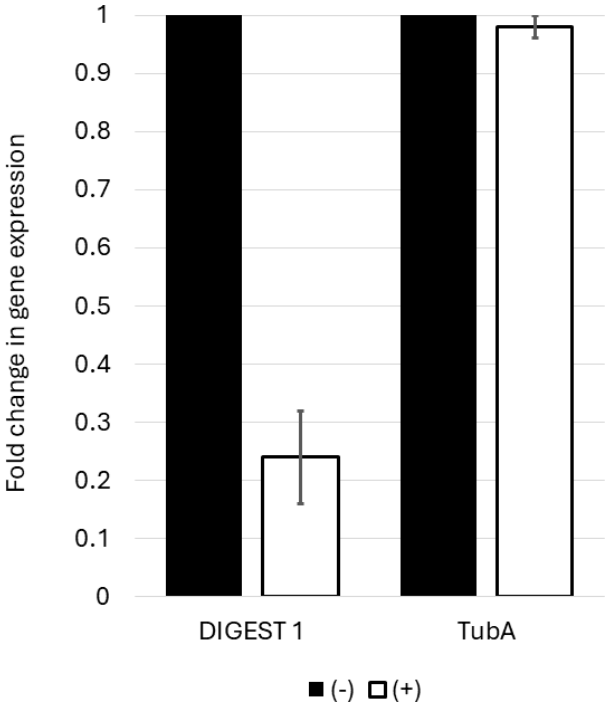

Figure 1. The relative change in gene expression for southern pine beetles sprayed with DIGEST1-targeting short hairpin RNA (shRNA) or mock treatment (n = 80). TubA, a cytoskeleton protein, was used as control.

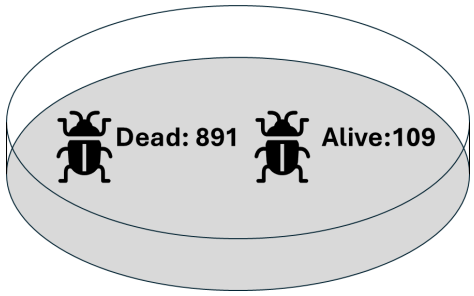

Figure 2. The survival of SPBs treated with DIGEST1-targeting shRNA at 5 days.

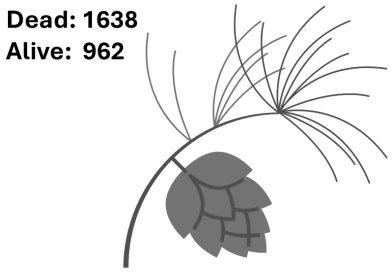

Figure 3. The survival of southern pine beetles after introduction to a pine leaf sprayed with shRNA at 5 days.

Table 1. Survival rate of beetle populations 15 days post treatment.

	Hemlock		*Pine*	
Population	(+) shRNA	(–) H_2O	(+) shRNA	(–) H_2O
Southern Pine Beetle	70.7 %	96.7 %	41.8 %	98.9 %
Mountain Pine Beetle	87.9 %	98.9 %	85.7 %	98.6 %

Meeting Summary

Tribal leaders asked us to reflect on the fact that the southern pine beetle has natural predators such as woodpeckers and other larger beetle species. The leaders emphasized a concern that our data show that the mountain pine beetle species is also affected and therefore the use of this technology may affect other important beetle species in the area. Certain beetle species are sacred to the land, while others are important food sources. The tribal leaders suggested that we enlist some of the community members to survey the beetle populations after spraying and report any concerns to our lab and the USDA.

Questions

1. What was the hardest part about writing to your audience? Why?

2. Why do you think that the federal government has placed importance on including ITEK in their future conservation strategies?

3. Historically, ITEK and Western practices have been separated in research efforts. How do you think the combination of ITEK and Western practices improve the understanding of our world?

4. Working with a community you are not familiar with can be a huge barrier to successful communication. How can scientists gather information about their audience's background, knowledge level, and interests to tailor their communication effectively?