Bacteria to Brains in Backyard Coyotes: An Interdisciplinary Pedagogical Case Study

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Abstract

Our interdisciplinary pedagogical case study explores the differences between rural and urban coyotes at the levels of organismal and community ecology. The focus is on how coyotes' gut microbiomes could affect their behavior via changes in the immune, endocrine, and nervous systems. The health and fitness of rural and urban coyote populations vary dramatically. Urban coyote health is poor as a result of their consumption of carbohydrate-rich anthropogenic food, compared to the natural protein-rich diet found in natural food sources. This case explores how altered microbiomes resulting from differences in diet can influence behavioral changes through the gut-brain axis, involving multiple physiological systems. The case showcases the interdisciplinary nature of science by having students explore the connection between macro- (whole organisms and communities of organisms) and micro-level (cellular and molecular interactions within an organism) systems. The case study is designed for introductory biology undergraduate students but can be adapted for more advanced and subdiscipline-focused courses within the life sciences.

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Learning Goals

Students will:

◊ understand the value and importance of making connections between subdisciplines within the life sciences that span immunology, microbiology, ecology, and animal behavior.

◊ From the Cell Biology Learning Framework:
- » How do cells send, receive, and respond to signals from their environment, including other cells?
- » How do cells connect to each other and organize to function as a collective entity?

◊ From the Ecology Learning Framework:
- » How do species interact with their habitat?
- » How are living systems interconnected and interacting?
- » What impacts do humans have on ecosystems?

◊ From the Immunology Learning Framework:
- » Layers of inducible and continuously present defense mechanisms resist, reduce, eliminate or tolerate antigens.
- » Homeostasis of the immune system can be altered or restored due to genetic or environmental influence.

◊ From the Microbiology Learning Framework:
- » How do microorganisms, cellular and viral, interact with both human and non-human hosts in beneficial, neutral, or detrimental ways?

Learning Objectives

Students will be able to:

◊ identify environmental factors (abiotic and biotic) that affect the distribution of a species.

◊ identify various types of interactions among coyotes and other species.

◊ outline the gut-brain axis and describe how it integrates multiple physiological processes.

◊ describe how the gut-brain axis can be influenced by environmental factors.

◊ construct a conceptual model for how coyote ecological context (urban vs. rural) can lead to changes in animal behavior and health.

◊ discuss the interdisciplinary nature of science.

INTRODUCTION

Although advances in immunology over the past decade have brought the field into the public spotlight, especially in terms of applications in professional health, integration of immunological concepts within undergraduate life science curricula are often lacking (1). To address this curricular gap, the NSF-supported ImmunoReach Research Coordination Network (2, 3) has set out to foster the generation of interdisciplinary curricular learning resources. The aim of these resources is to showcase approachable immunological concepts at a level that can be integrated into a variety of introductory life science courses. Here, two life science faculty from different disciplines, brought together through ImmunoReach, have used our expertise to generate an immunology-centered active learning classroom resource. Intended for an introductory biology classroom, the activity emphasizes the interdisciplinary nature of science by bringing together concepts that span immunology, microbiology, ecology, and animal behavior. This pedagogical case study was used to apply concepts and knowledge across traditional boundaries as called for within the core competencies outlined in the 2011 AAAS *Vision and Change in Undergraduate Biology Education* Report while showcasing immunological concepts (4, 5).

The case study explores differences between rural and urban coyotes at the levels of organismal and community ecology, including how coyote gut microbiomes could affect coyote behavior via changes in the immune, endocrine, and nervous systems. The health and fitness of rural and urban coyote populations vary dramatically with the latter being poor partially as a result of their consumption of carbohydrate-rich anthropogenic food, compared to the natural protein-rich diet (6). The case investigates how diet-altered gut microbiomes can influence behavioral changes (*e.g.,* aggression) of coyotes through the gut-brain axis involving multiple physiological systems. This case also showcases the interdisciplinary nature of science by having introductory biology students explore connections between macro- (organisms and communities) and micro-level (cellular and molecular interactions within organisms) systems and the scientists who carry out the research. Furthermore, this case study promotes immune literacy (7) through the adoption of a socio-scientific approach (8) to engage and expose introductory life science students to immunological concepts in the context of societal interactions with wild animals. The case purposefully exposes students to a minimal level of immunological jargon and avoids technical details inappropriate for an introductory level life science course (9).

Organismal and Community Ecology, Focusing on Coyotes

Ecology is so complex that scientists divide it into an ascending hierarchy of organismal, population, community, and ecosystem ecology (sometimes with additional levels) to make it more manageable.

Organismal ecology refers to adaptations of the individual to its environment and may include aspects of anatomy, physiology, and behavior. Here we focus on the last.

Behavior includes everything an animal does or does not do to find food, water, shelter, and mates or protect itself from predators (10). A common question about behavior is why an animal does what it does (11, 12) with two categories of causation: proximate, the more immediate causes of the behavior (including the gut microbiome and the associated gut-brain axis), and ultimate, causes having to do with its adaptive value (for survival/reproduction) and evolution.

Coyotes survive in their environment due to their heavy winter fur coats, excellent olfactory capabilities, ability to run fast and far, omnivory (eating everything from berries to insects to deer), and hunting prowess singly or in small packs (usually just a mated pair and their young). Their extremely varied diet is probably the main reason coyotes have been able to adapt to city dwelling. Studies of urban coyote diet show these animals eat fast food, fast food wrappers and other trash, fruits of ornamental plants (*e.g.,* figs, grapes), and domestic cats (outdoor pets or feral) (13–17).

A community is two or more interacting populations, and a major aspect of community ecology is the types of interactions that can occur. Some of these can be symbiotic (involving one species living in or on the other).

Coyotes experience a range of interactions, including competition with mesopredators (medium-sized predators) such as bobcats, foxes and even domestic cats (17–19); predation by mountain lions and wolves (18); cooperative hunting with badgers (20); parasitism by fleas, ticks, and worms; and colonization by pathogenic and non-pathogenic microbes (bacteria, fungi, and viruses, including rabies) (18). Just like the human gut, the coyote gut is full of commensalistic or mutualistic microbes, which is the focus of the rest of the case study.

The Importance of Mammalian Microbiomes

Macroorganisms are colonized by trillions of microbial cells living in diverse microbial communities alongside the host. This community of microbes is collectively known as the host's microbiome. A majority of these symbiotic microbes associate with the host in a mutualistic or commensalistic fashion and often can protect the host from establishment of parasitic infections (21). These symbionts influence mammalian fitness through metabolic byproducts that can modulate the immune and nervous systems (22, 23). Of mammalian microbiome body-site locations the gastrointestinal tract has been the most well studied and represents the largest surface between the host and the external environment (24). Cells of the immune system reside below the layer of gut epithelial cells and frequently sample antigens from the gut lumen to monitor for potential pathogens. Over developmental time, the host immune system learns to tolerate commensal host microbes. But when the composition of the microbiome dramatically shifts or is disrupted (*e.g.,* change in diet), a modulation in the immune response may result (*e.g.,* causing inflammation) (25).

The gut microbiome also plays a role in brain health by influencing the nervous system through production of neurotransmitters, neuro-active microbial metabolites including the short-chain fatty acids, and microbiome-stimulated immune system molecules such as cytokines (26). Cross-talk between the gut and brain through neuronal, endocrine, and immunological pathways thus influences neuronal development, brain chemistry, and animal behavior

(27–29); this bidirectional signaling is collectively referred to as the gut-brain axis (30, 31). Documented microbiome-influenced issues in humans include stress, anxiety, depression and diminished cognitive function, which all may directly influence behavior (29). For simplicity this case study focuses on the gut to brain direction of this two-way signaling street, but the central nervous system can also work in the other direction and influence the gut microbiome. For example, stress is associated with modulating the secretion of endocrine mediators in the gut lumen, which also bind to microbial receptors and influence microbiome composition (32).

Although mammalian microbiomes are resilient to a point, external factors (*e.g.,* diet, drugs including antibiotics) can influence gut microbiome composition (33, 34). These external perturbations can transform microbiome composition from host-microbial homeostasis (eubiotic state) to an imbalance of gut microbiome composition referred to as a dysbiotic state. Dysbiosis can in turn modulate immune, endocrine, and neurological systems. These physiological changes can influence organism fitness (*e.g.,* susceptibility to disease, change behavior).

This lesson allows students to see the interconnections of diet, the microbiome, gut-brain axis physiology, and behavior in coyotes.

Intended Audience

We taught this lesson in introductory biology classes taken by biology majors and minors at two small liberal arts colleges. One course focused on cell biology; it included the lesson after exposing students to the concept of cell differentiation coupled with an exploration of the structure and function of neuronal and endocrine cells. The other course focused on ecology, evolution, and diversity (with an introductory cell biology and genetics course as the prerequisite). Here we scheduled the lesson after organismal and community ecology (the former of which included an introduction to animal behavior).

Required Learning Time

This lesson is designed to be taught over a single 50-minute class period. Students are also assigned a ~30-minute pre-class preparation activity and a ~30-minute post-class reflection activity. See Table 1 for recommended timing of individual case study elements within the class period.

Prerequisite Student Knowledge

The case is designed with an introductory life science major in mind, but could be implemented in a non-majors life science course. The case was successfully implemented in introductory cell biology and ecology courses with diverging course learning outcomes. For a course focused on cell and molecular biology it is suggested that students be exposed to the concept of cellular differentiation and specialization in multicellular eukaryotes. For a course focused on ecology, faculty who wish to incorporate content on proximate/ultimate causes of behavior should have covered these concepts prior to the case. Students should also have some understanding of resources, including biotic factors such as food sources, and abiotic factors such as weather, that affect animal distributions and community interactions among a variety of species.

Prerequisite Teacher Knowledge

We recommend that the instructor be familiar with ecological and cell biology concepts at a level that is appropriate for the course in question. This would include environmental factors that affect animal distributions (including coyote range expansion across the U.S. in the last 150 years), types of community interactions (predation, competition, parasitism, etc.), and optionally proximate and ultimate causes of behavior. Cell and molecular biology concepts include cellular differentiation and specialization in multicellular eukaryotes, structure and function of specialized cell types, an abridged overview of the immune system and immune response, paracrine and endocrine signaling, cytokines, and eubiotic and dysbiotic states of microbiota. Considering the interdisciplinary nature of this case study we have put together a brief synopsis including references that adopting instructors may find useful (Supporting File S1). Instructors should also have some familiarity with active learning methods such as case study teaching (35).

SCIENTIFIC TEACHING THEMES

Active Learning

Use of pedagogical case studies in the classroom involves a special type of educational storytelling to engage students in active learning through discussion (36). Case studies provide a situation or a problem delivered through an engaging narrative for students to analyze and apply course concepts to (37). The pedagogical process then provides students with a structured instructor-facilitated environment to dive into the details and discuss the case with peers (38). Case studies such as this face-to-face resource can also be adapted for implementation across diverse classroom modalities (39). Use of case studies within science education has been advocated as an active learning technique to replace passive lectures by encouraging students to learn by doing (35, 36).

Assessment

Integrated assessment questions within the case study were designed to promote student critical thinking and dissect complex cellular and molecular processes. Instructors are encouraged to use the integrated case questions for just-in-time style formative assessment (40) during in class discussions of the case concepts. Part I and III integrated formative assessment questions allow the instructor to follow-up and debrief students during the subsequent class period after being assigned, as well as to engage students outside of class and help prepare students to dive into the meat of the case study (Part II).

Additional multiple-choice and open-response summative assessment questions allow the instructor to assess student learning through quizzes and/or exams (Supporting File S2). Furthermore, students can self-evaluate their own learning though a post-activity perspective-style survey (Supporting File S2). Institutional Review Board (IRB) approval to collect and disseminate anonymous student assessment data was provided by the University of Dubuque IRB protocol #1060.

Inclusive Teaching

The interdisciplinary nature of the topic of our case study as well as the interaction between the two characters (Ken and

Aisha) illustrate the importance of having diverse voices in a conversation. Ken, an immunologist, and Aisha, a behavioral ecologist, learn a great deal from each other. We hope they also serve as potential role models for students who would not have considered research careers. Although not made explicit, we imply different racial or ethnic backgrounds of these characters by our choice of their names. Having a female character studying coyotes in the field may also expand the perception of gender roles for some students. During the activity, students work independently and in groups, discussing and writing about the topic. The possible extensions to the activity allow students the autonomy to choose their own topics of exploration (described in the *Teaching Discussion* section). The case study thus meets several recommendations of Universal Design for Learning (41) including diverse means of accessing information, sharing learning, and engagement with the activity.

LESSON PLAN

There are three components to this case study: (i) Part I: Pre-class preparation, (ii) Part II: Classroom activity, and (iii) Part III: Post-class reflection (Table 1). Prior to implementation, the instructor should work through the case study (Supporting File S3) and review the suggested solutions for the integrated formative assessment questions (Supporting File S4).

Part I: We asked students to read the introduction portion of the case study (about 3 pages) and answer the two reflection questions included there before coming to class (Supporting File S3). This should have taken less than 30 minutes. One of us also assigned a brief article from the local newspaper highlighting regional coyote populations living in the rural/urban interface; the other projected and quickly summarized the article at the beginning of class.

Part II: Class began with a quick discussion of the assigned reflection questions associated with Part I of the case study. This discussion was followed by connecting the topic to local coyotes through debriefing on a popular press article about regional coyote populations to promote engagement through place-based learning (42). This article was provided as part of the pre-class assignment by one instructor and simply summarized during the class session by a second instructor. Students were guided to work in groups to complete Part II of the case study in the remainder of a single 50-minute class period with the instructor bringing the class together to debrief. One of the final formative questions prompts student groups to predict outcomes based on their knowledge from the case to model how the gut microbiome and behavior are associated. Depending on the focus of the course, instructors may include or exclude the optional question on proximate/ultimate behavior. This question was explored when implemented in an introductory ecology class but excluded when implemented in an introductory cell biology course. See "Background on Ecology" section within Supporting File S1 for more information on proximate/ultimate causes of behavior.

Part III: The final portion of the case study consists of two formative assessment questions assigned as out-of-class homework to allow students to reflect on the concepts explored during class. Students probably completed this homework in under 30 minutes and submitted their responses through the course learning management system or on paper.

TEACHING DISCUSSION

We implemented the case study with four independent cohorts of students across two academic years. This included an introductory biology course focused on ecology and an introductory cell biology course.

Effectiveness in Meeting Learning Objectives and Student Reactions

The case study was formally assessed using author-generated summative assessment questions and through a retrospective student perceptual survey (Supporting File S2). Students overwhelmingly expressed their belief that the case study format was more conducive to learning about the gut-brain axis and interdisciplinary nature of science as opposed to a standard lecture (Figure 1). We also observed marked increases in students' retrospective perception of their own learning for Likert-scale items stating that they can confidently meet each of the learning objectives of the case study (Figure 1).

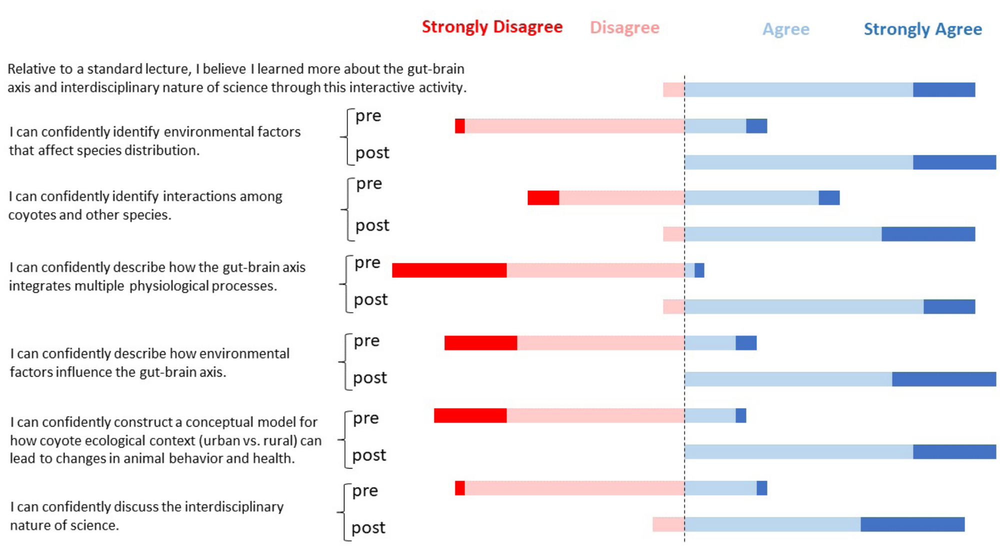

Figure 1. Retrospective post-pre student perceptions survey data. Data was collected from the original pilot implementation at the two participating institutions with an aggregate n = 30.

Thematic analysis of open-response comments on students' holistic perception of the case study indicated positive sentiment that touched on three major themes (i) place-based learning, (ii) student engagement, and (iii) the interdisciplinary nature of science (Table 2). Student quotes that touched on place-based learning specifically highlight how students perceived the impact of instructors drawing from local popular press articles (*e.g.,* newspaper) to underscore that the case study subject was of local regional importance.

Author-generated summative assessment questions were integrated into course unit exams or quizzes. Overall, students performed well on summative assessment items (Table 3). A multiple-choice question focused on the concept of microbiome dysbiosis was flagged due to low student performance during the initial pilot. Point-biserial item analysis of the flagged question resulted in a negative value suggesting the students may have had issues in question interpretation (43). In our second iteration of case study implementation and assessment, we created an alternative microbiome dysbiosis question, which performed well with a point biserial index in the acceptable range.

Possible Extensions

If instructors wish and time permits, our case study could be used as a jumping-off point for students to further explore topics such as animal behavior, community ecology, immunology, and microbiology. Specifically, the case ends with an opportunity for instructors to extend the conversation around immigration, gut microbiome, and health. Extensions could also highlight the interdisciplinary nature of the natural sciences through a choose-your-own-adventure-style activity. In courses such as general biology, students are typically heterogeneous in their life science subdiscipline interests (*e.g.,* community ecology, animal behavior, microbiology, and immunology). Students may sign up to perform further research with some supplemental reading on a subdiscipline represented in the case study. Students would be instructed to write a summary outside of class or prepare a presentation. A great deal of research exists on microbiomes, the gut-brain axis, and changes in behavior resulting from altered microbiomes. In Supporting File S1 we recommend primary and secondary sources for students to explore if they wish to engage these topics further. Students could share their new learning with peers in a jigsaw (44) or gallery walk (45) during an additional class period.

Although the gut-brain axis is bidirectional in nature, the case study focuses on the gut to brain direction for simplicity. Another avenue for activity expansion would be for students to investigate how the brain can influence the gut microbiome (46).

SUPPORTING MATERIALS

- S1. Bacteria to Brains – Literature Review for Instructors
- S2. Bacteria to Brains – Assessment Instruments
- S3. Bacteria to Brains – Case Study Handout
- S4. Bacteria to Brains – Case Study Answer Key

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Table 1. Case study implementation summary and timeline.

Activity	Description	Estimated Time	Notes
Pre-Class Preparation			
Introduction to the case study	Students read Part I of the case and answer the two reflection questions it contains.	20 minutes	The complete case for students is available in Supporting File S3. Instructors may want to consider separating Parts I, II, and III so that each part can be passed out sequentially.
Local connection	Optional but recommended: find an article local to your area discussing coyote populations. (There were two in our small-city newspaper in the last year, including one on why the coyote population is expanding and recommending not to feed them!) Ask students to read the article and be prepared to share thoughts in class.	10 minutes	See alternative "local connection" activity in the "Classroom activities" section" below
Classroom Activities			
Local connection	Ask students for their reaction to the local news article. If this was not shared in advance, project an article for the class and summarize it briefly, making the point that coyotes are present and interacting with people even in your area.	3 minutes	
Discussion of pre-class homework	**Case Study Part I:** Ask students to briefly share their answers to questions 1 and 2 in the case study.	5–10 minutes	Suggested answers to all case study questions are included in Supporting File S4.
Continue the case study	**Case Study Part II:** Have students complete the second part of the case study, discussing their answers in small groups. Circulate around the room to eavesdrop and answer questions they have. It may be helpful to interrupt once most students have reached Figure 3 and go over it with them briefly, emphasizing the explanation on the right-hand side and how it matches the image in the center (project the image so everyone sees the specific connections being discussed). Emphasize the terms "eubiosis" and "dysbiosis" for those students who delve into the details and miss the big picture. Metabolite might be a term your students don't know (depending on course content), so be sure to define it in connection to the figure. Ask groups who are a little further ahead to share their answers to questions 4 and 5 to make sure they are on the right track and help other students along.	30–40 minutes	Question 8 is optional depending on course content
Wrap-up	Answer any questions in a whole-class discussion. Sketch the scenarios in question 9 on the board and ask students which way the arrows should go. While doing this, ask students to define terms from the figure such as metabolites and dysbiosis and explain why the arrows point the way they do.	7 minutes	
Post-Class Reflection			
Assign homework	**Case Study Part III:** Questions 10 and 11 are intended as homework.	30 minutes	
Summative Assessment			
Exam questions	Include the suggested multiple-choice and short-answer questions as part of the next class assessment.	5–10 minutes of exam time	These questions are included in Supporting File S2.

Table 2. Selected students' verbatim post-survey perceptional quotes.

Theme	Exemplar Quote(s)
Place-based Learning	"I thought it made me think critically about a subject that is relevant to the Dubuque [Iowa] area that I had never thought about before so that was cool."
Engagement	"It was one of my favorite case studies so far this semester." "I enjoyed how the scenario was like a conversation and slowly lead us through the information that needed to be know. It described most of the terms I did not know. I really enjoyed this case study."
Interdisciplinary Nature of Science	"I enjoyed the demonstration of two scientists from different fields educating each other." "I enjoyed learning about how small processes and organisms such as the microbes in the coyote affect larger processes and organisms such as the health of the coyote and its relationship with its environment."

Table 3. Student summative assessment performance on quiz and examination items*.

Learning Objective	*N*	Average Score (Mean % ± SEM)	Point Biserial Index**
Outline the gut-brain axis and describe how it integrates multiple physiological processes. **(focus on understanding of cytokines)**	76	73.7 ± 0.051	0.322
Outline the gut-brain axis and describe how it integrates multiple physiological processes. **(focus on understanding of gut-brain axis)**	76	77.6 ± 0.048	0.324
Describe how the gut-brain axis can be influenced by environmental factors. **(focus on understanding of dysbiosis) - version 1.0 (2022)**	37	27.0 ± 0.073	-0.012
Describe how the gut-brain axis can be influenced by environmental factors. **(focus on understanding of dysbiosis) - version 2.0 (2023)**	39	82.1 ± 0.061	0.464
Construct a conceptual model for how coyote ecological context (urban vs. rural) can lead to changes in animal behavior and health.	60	89.9 ± 0.063	0.418

*Aggregate of two separate courses at independent institutions

**A minimum acceptable correlation coefficient threshold of 0.15 is suggested, with good items generally performing at >0.25 (43).

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