ISSUES : FIGURE SET

Using functional responses to investigate the ecological consequences of an introduced biological control agent

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THE ISSUE

Biological control agents are used in a wide range of contexts to limit damage from pests. However, the broader ecological consequences of such agents often are unclear before ecological risk assessments are performed. This Figure Set guides students to think through potential consequences of using biological control agents, and then uses a specific study to challenge students to interpret results from laboratory and caged field experiments. This Figure Set also introduces the concept of functional responses to students.

ECOLOGICAL CONTENT

biological control agents, functional responses, predator-prey relationships, ecological risk assessments

STUDENT-ACTIVE APPROACHES

Think-pair-share, drawing predicted results, designing experiments

STUDENT ASSESSMENTS

answering questions on a worksheet, sharing responses with the class, and completing post-class homework that assesses understanding of key concepts

OVERVIEW

WHAT IS THE ECOLOGICAL ISSUE?

Biological control agents, organisms released in order to control pests, are often promoted as an alternative to the use of pesticides, which are seen as damaging to both the environment and human health (Louda et al. 1997). However, the release of such biological control agents can have unintended consequences on the target organism and local ecosystems (Reilly and Elderd 2014). In particular, releases of such biological control agents can influence non-target species, detrimentally impacting not only populations of the target pest but those of other non-targeted organisms co-occurring in the same area as the target organism

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(Louda et al. 2003). Given this, it is critical to understand how such biological control agents may influence the broader ecosystem before the release of such control agents. In addition, this issue is of particular concern given that the impact of biological control agents on non-target organisms is expected to increase as global climates continue warming (Lu et al. 2014).

This topic is also highly relevant as it presents an issue at the intersection of ecology and society. Students may already be familiar with this issue given the frequent coverage of biological control agents in the news and popular media. For instance, Google's recent release of two million sterile, non-biting mosquitoes to control the spread of Zika and other mosquito-borne diseases was covered in major media outlets (e.g., May 2017, Wang 2017), and local newspapers often have articles covering local uses of biological control agents.

Students will explore this issue through this Figure Set by thinking critically about functional responses, another important ecological concept. Functional responses define the relationship between the rate of a given consumer eating its food as compared to the density of its food, and can be classified as type I, type II, or type III responses depending on how the rate of consumption changes as the density of the food source changes (Holling 1965). Understanding such functional responses is critical not only for assessing the impacts of biological control agents, but also for analyzing a range of other ecologically relevant behaviors such as foraging and predation (Durant et al. 2003, He et al. 2012).

FIGURE SET TABLE

Figure Set and Ecological Question	Student-active Approach	Cognitive Skill	Class Size/Time
Non-target impacts of the biological control agent <i>Harmonia</i> <i>axyridis</i> on <i>Danaus</i> <i>plexippus</i>	Experimental design, think-pair- share, interpreting data and results	Know, comprehend, interpret, analyze, synthesize	Any/long
(Koch et al. 2003)			

FIGURE SET BACKGROUND

Harmonia axyridis, known as the Asian ladybeetle or Asian ladybug, has been used as a biological control agent with increasing frequency for at least the past two decades (Koch 2003). Native to Asia, the species was first introduced to North America in the 1980s and has spread across the continent as well as to

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South America and Europe (Brown et al. 2008; Koch 2003; Koch et al. 2006). The species preys upon aphids and other agricultural pests. However, the broader ecological consequence of these introductions, and in particular, the impact on non-target species, was only investigated decades after the first recorded use of ladybeetles as biological control in the late 1800s and the first use of *H. axyridis* in 1916 (Roy and Wajnberg 2008). Indeed, the use of *H. axyridis* as a biological control agent has led to many unintended consequences, including its decimation of populations of native aphid-eating species, its consumption of certain agricultural fruits, and its high aggregations in human-dwelling areas (Koch et al. 2004; Kovach 2004; Majerus et al. 2006; Roy and Wajnberg 2008). Consequently, the species is now itself considered a pest (Kovach 2004; Roy and Wajnberg 2008).

Here, students will explore a series of laboratory and caged field experiments published in 2003 to investigate if *H. axyridis* is having a specific impact on monarch butterflies (*Danaus plexippus*). Monarchs are a charismatic species that many students will immediately recognize; the species is also widely perceived as an icon for conservation (Gustafsson et al. 2015). Thus, this study focuses on two common insect species that will be immediately familiar to many students, which should help facilitate understanding and discussion of the experiments. This study also allows for a broader discussion of monarch butterfly conservation following this Figure Set, as monarch populations worldwide continue to decrease due to habitat loss and climate change exacerbated by anthropogenic activities (Inamine et al. 2016; Vidal et al. 2013).

In addition to investigating non-target impacts, this Figure Set will also introduce the concept of functional responses to students. The study features four experiments, depicted in three figures. The first set of experiments are done in the laboratory and test the functional response of adult or third instar (larvae) *H. axyridis* feeding on either *D. plexippus* eggs or first instar (small, immature larvae) *D. plexippus*. The number of *D. plexippus* provided was varied in these experiments, and the amount of prey consumed within 24 hours was recorded. Through guided inquiry questions, students will predict trends, analyze the data, and also compare and contrast different types of functional responses. The second set of experiments was conducted in the field, with researchers tracking the survival of *D. plexippus* over time in a cage with varying numbers of *H. axyridis* placed in the cage.

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FIGURES

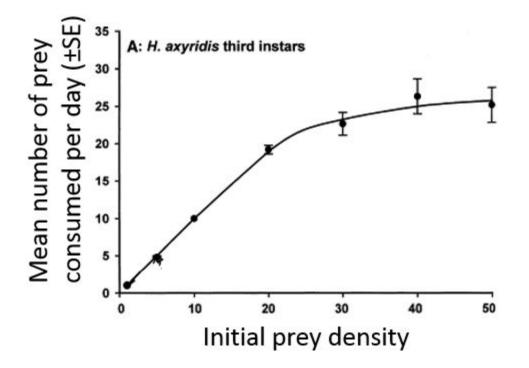


Figure 1: A. (incomplete) Relationship between initial *D. plexippus* egg density and the number of *D. plexippus* consumed per day by a single *H. axyridis* larva. Based on Koch et al. (2003). Reprinted from Biological Control, Volume 28, R.L. Koch,W.D. Hutchison,R.C. Venette,G.E. Heimpel, Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae), Copyright 2003, with permission from Elsevier.

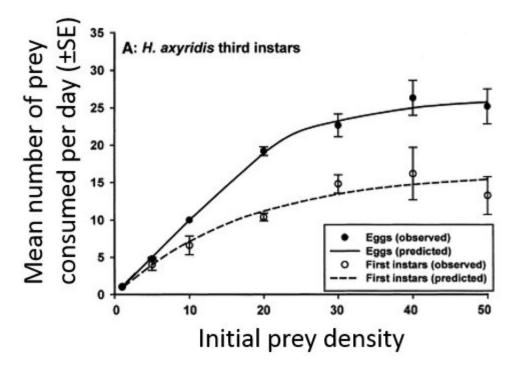


Figure 1 A. (complete) Relationship between initial *D. plexippus* egg or small caterpillar densities and the number of *D. plexippus* consumed per day by a single *H. axyridis* larva. Based on Koch et al. (2003). Reprinted from Biological Control, Volume 28, R.L. Koch,W.D. Hutchison,R.C. Venette,G.E. Heimpel, Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae), Copyright 2003, with permission from Elsevier.

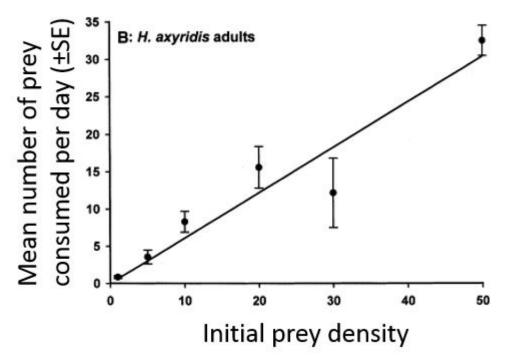


Figure 1 B. Relationship between initial *D. plexippus* egg densities and the number of *D. plexippus* eggs consumed per day by a single adult *H. axyridis*. Based on Koch et al. (2003). Reprinted from Biological Control, Volume 28, R.L. Koch,W.D. Hutchison,R.C. Venette,G.E. Heimpel, Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae), Copyright 2003, with permission from Elsevier.

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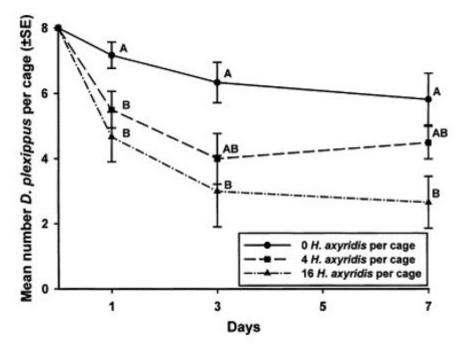


Figure 2. Survival of *D. plexippus* in caged field experiments with varying numbers of *H. axyridis* per cage. Based on Koch et al. (2003). Reprinted from Biological Control, Volume 28, R.L. Koch,W.D. Hutchison,R.C. Venette,G.E. Heimpel, Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae), Copyright 2003, with permission from Elsevier.

Background Information

Biological control agents are organisms released in order to control pests. Such agents offer an important alternative to pesticides, which can be damaging to both environment and human health. These biological control agents can help prevent pests from destroying agricultural crops, or even help limit the spread of disease among humans. For instance, you may have heard about Google's recent (July 2017) release of two million sterile, non-biting mosquitoes in California to control the spread of Zika, an infectious disease that has had multiple recent outbreaks.

In class, you will be learning more about biological controls and will be thinking through the ecological implications of using such controls. First, however, it is important to understand the broad range of what constitutes biological control. For your **pre-class assignment**, find a recent (from the past year) news article that features biological control and is not the same example as the one mentioned above. Read this article, bring in a copy for class, and be prepared to share your article with your peers. We will use your articles to illustrate the diversity of biological control and the importance of understanding such ecological principles.

During class:

We have explored a range of biological controls in the news and will now focus on a specific example. *Harmonia axyridis*, or the Asian ladybeetle (alternatively known as the Asian ladybug), was first used in the 1900s as a form of biological control, where organisms are introduced to control the spread of pests. The species preys upon aphids, a destructive agricultural pest. Native to Asia, the *H. axyridis* was first introduced to North America in the 1980s. However, the introduction of this species has produced many unintended consequences, including the decimation of many native species of insects in North America, as well as high aggregations of the ladybeetle becoming a human nuisance in many cities. Consequently, this Asian ladybeetle has itself been labelled as a pest! In this module, you will investigate what impacts, if any, this species has on the monarch butterfly, *Danaus plexippus*, a charismatic insect native to North America.

Part I:

In this study, scientists were interested in examining potential non-target impacts stemming from the introduction of *H. axyridis* as biological control. In other words, they wondered if *H. axyridis* has the potential to impact populations of other organisms besides the aphids they were meant to help control. More specifically, they were interested in seeing if *H. axyridis* adults or larvae

(described as being in the third instar, a stage of development) could be preying on the eggs of the monarch butterfly (*D. plexippus*) or eating immature monarch larvae (small caterpillars).

- Design an experiment, and sketch your design below, that determines the potential impact of *H. axyridis* adults or larvae on *D. plexippus* populations. Be sure to note what your independent and dependent variables are, and what conditions might be controlled in your experiment.
- 2. Sketch a revised experiment after discussing with your group.

<u>Part II:</u>

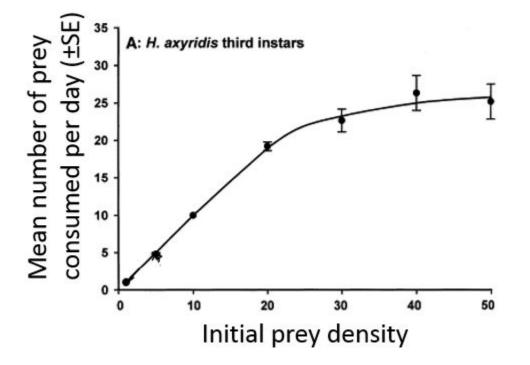
The authors of this study conducted two sets of experiments. The first set of experiments was conducted in the lab, where they placed 1, 5, 10, 20, 30, 40, or 50 monarch eggs in a petri dish, and then introduced a single *H. axyridis* larva that had been starved. They then observed how many monarch eggs were eaten over a 24-hour period, and repeated this experiment several times. For each series of experiments conducted with the same initial number of monarch eggs, they calculated the average (mean) number of monarch eggs eaten in these experiments by the single *H. axyridis* larva, which thus represents an estimate of the predator's per capita rate of consumption.

3. Predict what you think the results looked like by sketching in the graph below. Explain your prediction.

Number of monarch eggs eaten by single *H. axyridis*

Number of monarch eggs placed in petri dish

Now, examine the actual results from the experiment.



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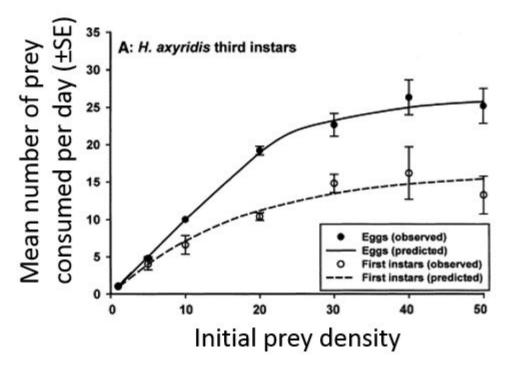
- 4. Was your prediction accurate or not? If not, what surprises you about the actual results?
- 5. Why do you think the number of eggs eaten after 24 hours started to plateau off after a certain number of monarch eggs was made available?

This graph shows a **functional response**, which charts the predator's per capita rate of consumption depending on the initial density of prey or vegetation. Functional responses can be classified into three specific types; the graph you see here represents a **Type II functional response** where the **rate** of prey or vegetation eaten remains constant at low prey or vegetation densities, gradually decreases as the density of prey or vegetation increases, and then eventually asymptotes at a high prey or vegetation density.

The second experiment was extremely similar to the first experiment, but offers the single *H. axyridis* larva **monarch butterfly larvae** (small caterpillars) instead of monarch eggs as food.

6. Using Figure 1A above, draw in a dashed line to predict what you think this functional response will look like. Explain your reasoning.

<u>Part III</u>



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Compare the two functional responses of *H. axyridis* larvae feeding on monarch 1) eggs and 2) larvae.

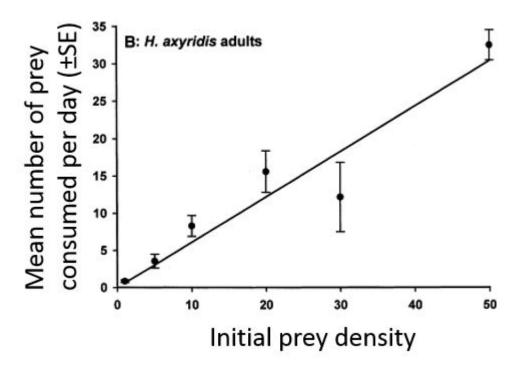
- 7. Does the functional response of *H. axyridis* larva on monarch larvae also fall under type II? Why or why not?
- 8. Why do you think there is a difference in the functional response graph between *H. axyridis* feeding on monarch eggs and larvae?
- 9. Based off these data, are monarch eggs or larvae more susceptible to *H. axyridis* larvae?

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<u>Part IV</u>

In the third experiment, the researchers now investigated the functional response of **adult** *H. axyridis* on *D. plexippus* eggs by measuring the amount of *D. plexippus* eggs consumed by a single *H. axyridis* adult in a day.



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10. Why do you think that this functional response does not asymptote?

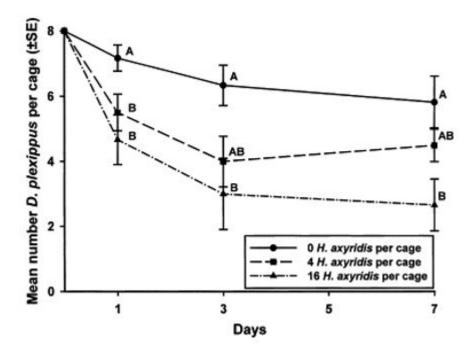
This trend, if it holds under further experiments with increased numbers of prey, is known as a **Type I functional response.** A type I functional response is

characterized by a **linear relationship** between the density of prey or vegetation and the amount of prey or vegetation consumed.

- 11. Look carefully at the graph again. Why do you think there is a recorded **decrease** in the mean number of *D. plexippus* eggs eaten when increasing the number of eggs available from 20 to 30?
- 12. How might you test your ideas from the previous question?
- 13. Contrast the differences in impact on the **prey populations** between a type I and type II functional response. Would predators exhibiting type I or type II functional responses be more effective at controlling prey populations?

<u>Part V</u>

The last experiment in this study was conducted in the field, where researchers set up several cages to control how many *H. axyridis* larvae and *D. plexippus* larvae would be present in the system. The cage ensured that no other individuals would be able to enter or exit the system. Each cage started with eight *D. plexippus* larvae, and the researchers set up cages with no *H. axyridis* larvae, 4 *H. axyridis* larvae, and 16 *H. axyridis* larvae. They then tracked each system for a week, recording the number of *D. plexippus* larvae surviving in the cage.



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- 14. What was the purpose of including a cage with **no** *H. axyridis* present?
- 15. What additional insight did this experiment provide over the previous experiments conducted in the lab?

<u>Part VI</u>

Now that you have seen the results from each of the experiments, we will consider the data together to infer conclusions.

- 16. What conclusions can you draw from these experiments? Do you think that *H. axyridis* may have non-target impacts on *D. plexippus*? Explain.
- 17. Are there possible broader ecological implications or consequences (beyond these two species) that you can draw from this experiment? Explain.
- 18. How realistic do you think these experiments are in reflecting natural populations?
- 19. What other information would you want in order to determine the ecological impact of *H. axyridis* on *D. plexippus*?
- 20. What follow-up experiments would you want to complete to investigate this question further?
- 21. How did the experiment you designed in part I compare to the experiments presented here? What strengths and weaknesses do you see in the experimental design for your experiment versus the study's experiments?

Post-class assessment:

Please complete the following questions for homework.

- 1. In your own words, explain how we know that *H. axyridis* may have an impact on *D. plexippus*. Be sure to provide a complete explanation so that someone who has not seen this Figure Set is able to understand the experiments and data.
- 2. Research other non-target impacts of *H. axyridis*, and provide a brief paragraph summarizing what you found.
- In class, we discussed both Type I and Type II functional responses. There are also Type III functional responses. Do some research on type III functional responses, then draw a type III functional response and

describe the difference between each of the three types of functional responses.

NOTES TO FACULTY

The activity includes a pre-class assignment, where students are asked to find a news article that discusses biological control. This pre-class activity is designed to engage students in thinking about how ecological issues play a key role in society, and to pique their interest on this topic. Depending on class size and time allotted for this module, you may wish to have a general discussion on the ethics of using biological control agents, particularly genetically modified organisms. For large classes, you may have students share their articles with their neighbors and have smaller discussions, and for small classes, you may be able to have a class-wide discussion. Depending on your chosen format, you may wish to have students turn in their news articles to you before class so that you may review the articles and facilitate the discussion accordingly.

The in-class activity is divided into six parts, which should be handed out separately in sequential order. Parts III, IV, and V can be combined together and handed out in one packet if needed, but the other parts are written so that students should not have access to future parts while working on another section since there are multiple questions that ask students to make predictions and inferences before they see the actual figures and data. (Alternatively, you can also make a packet, put each part on a separate page, and ask students not to turn ahead to the next part until they are finished with the previous part.) There are 21 inquiry-based questions throughout these parts that challenge students to design experiments, interpret and assess graphs, and synthesize the information to draw conclusions. Before beginning these parts, you may wish to briefly introduce the study systems of *H. axyridis* and *D. plexippus*. Students should work in small groups to answer these questions.

Part I uses a think-pair-share and asks students to design an experiment to test if *H. axyridis* has an impact on *D. plexippus*. After students are done sketching their experimental designs, instructors should ask students to pair with other students and share their ideas. During this time, instructors should check in with groups to get a sense of students' experiments and to help guide the conversation. Note that this question is intentionally left open-ended in order to allow students creativity in designing experiments. Consequently, some students may design field-based experiments, while others may rely on lab-based experiments similar to what was performed in Koch et al. (2003). Instructors should focus on providing feedback on the experimental design of student experiments, ensuring that student-designed experiments are able to answer the

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posed question, are well-controlled with clear dependent and independent variables, and are feasible, and may also wish to mention these criteria for students to consider while they are answering question 2 in small groups. After students have had the chance to share within their small groups, instructors should facilitate a class-wide discussion of the experiments that students designed, and then introduce the design behind the first experiment included in Koch et al. (2003), which is covered in part II.

After reading about the first experiment, students are then challenged to predict the results in part II by filling in the graph in guestion 3. Most students will draw a linear relationship here (a Type I functional response) and justify it by saying that the more prey that are available, the more that the predator will eat, although some students may draw a Type II curve. Students are then asked to compare their prediction with the actual data in the study, and explain why they think the per capita rate of consumption begins to asymptote at a higher prey density (question 5). Students may respond that the single predator is "full" or "done eating", and instructors should use this opportunity to ask students to formalize their thoughts in more appropriate ecological terminology. In other words, instructors should guide students toward the concept that as the single H. axyridis becomes satiated it will thus decrease its rate of prey consumption. Students are then introduced to the concept of functional responses before they are asked to predict the functional response for the second experiment, where they will likely draw another Type II curve. Instructors may wish to survey the room to gauge student predictions and see how their predicted per capita rate of consumption of *D. plexippus* larvae varies as compared to the data for consumption of *D. plexippus* eggs. It is also recommended that instructors check in with the class at this point and explain the concept of functional response, and indicate that the future experiments will continue building upon this concept.

In part III, students interpret results from the second experiment and compare and contrast the functional responses between *H. axyridis* and *D. plexippus* larvae versus *D. plexippus* eggs. This new curve is still considered a Type II curve (question 7) since there is a decreasing rate of consumption as the density of prey increases. Despite this, it appears that starved *H. axyridis* larvae will eat more *D. plexippus* eggs than *D. plexippus* larvae, meaning that *D. plexippus* eggs are more susceptible than *D. plexippus* larvae (question 9). Students may provide a range of explanations for why this is (question 8), including potential differences in energy provided between a single *D. plexippus* egg and larva or differences in level of ease in obtaining the prey. Instructors may wish to ask students how likely they think their explanations are and how they might test their ideas to provide more practice for students in thinking about designing experiments.

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Students examine the last functional response graph in part IV. However, unlike the previous functional responses, we only see a linear trend between the density of prey and the amount consumed. This is presented as a possible Type I functional response, but some students may note (in question 10) that the trend may not stay the same if the authors had tested greater numbers of D. plexippus eggs being present. As such, if the rate of consumption begins to decrease with an increase in eggs, then this may become a Type II functional response, a possibility that the authors of the paper suggest is the likeliest outcome: "The linearity of the functional response of adult H. axyridis on D. plexippus eggs was likely due to the predators not being exposed to high enough prey densities to induce satiation" (Koch et al. 2003). The next two questions ask students to analyze why there might be a **decrease** in the number of *D. plexippus* eggs consumed when the number of eggs present is increased from 20 to 30, and to think of an experiment to test those ideas. Students will likely come up with a range of responses here, and instructors should encourage any idea that appears to be ecologically feasible. Like before, instructors should evaluate the merits of proposed experiments by ensuring that experiments are capable of answering the posed question, are well-controlled, and have clear independent and dependent variables.

Following this discussion, the instructors may wish to bring up how the authors of the paper (Koch et al. 2003) approached this decrease. Although this decrease may not be statistically significant, the authors attribute this change to the potential of area-restricted search, where the predator may temporarily slow down its intake after finding an area with high densities of prey:

There is a peculiar dip in observed predation by adult *H. axyridis* on *D. plexippus* eggs at the initial prey density of 30 eggs per dish. Heimpel and Hough-Goldstein (1994a) found a similar response for the stink bug *Perillus bioculatus* (F.) preying on neonate *Leptinotarsas decimlineata* (Say), and attributed the response to successful area-restricted search following subsatiation feeding at high prey densities (Heimpel and Hough-Goldstein, 1994b), but not at low densities.

The last question of this part asks students to explicitly consider how predators who exhibit type I or type II functional responses may differentially impact the respective prey populations. The previous questions have focused on asking students to think through type I and type II functional responses through examining the impact on the predators; this question thus is designed to encourage students to reflect on how such relationships impact the prey populations. The second part of this question asks students to consider now whether predators exhibiting type I or type II functional responses would be more effective at controlling prey populations. The effectiveness of such biological

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controls would depend on the precise per capita rates of consumption, but in general predators exhibiting a type I functional response would be more effective than predators exhibiting a type II functional response. This is because predators exhibiting a type I functional response would not see a decrease in per capita rate of consumption with increasing prey densities, as would be observed in predators exhibiting a type II functional response. Instructors may wish to emphasize this point and discuss the importance of understanding type I and type II functional responses when using biological control.

The last experiment, conducted in the field, is analyzed in part V. Students are asked about the value of running this experiment with no *H. axyridis* present, testing their understanding of controls, and then are challenged to think about what additional information this experiment provides over the previous set of experiments. Instructors may need to remind students that this is the only experiment done in the field with host plants, rather than being done in the lab in a petri dish like the earlier experiments. This distinction is important to point out for question 15, given that the previous series of experiment is the only one discussed here that attempts to replicate natural field conditions, while this last experiment is the only one discussed here that attempts.

The final section of the in-class activity, part VI, has students synthesize all the information presented to draw conclusions. After thinking about these conclusions in guestion 16 (that *H. axyridis* can prey on *D. plexippus* and that there thus is likely non-target impact on *D. plexippus* from introductions of *H.* axyridis as biological control), students are asked to think of the broader ecological significance of these conclusions (question 17). Instructors may wish to ask follow-up questions about what other organisms may be impacted by declining D. plexippus populations. For instance, D. plexippus feeds on milkweed (genus Asclepias); how might declining D. plexippus populations impact milkweed? Similarly, many ants, wasps, spiders, and various birds prey on D. plexippus; how might these predator populations be impacted if H. axyridis is introduced and now preys upon *D. plexippus*? Students might cite these potential corresponding increases in milkweed and decreases in *D. plexippus* predator populations as additional downstream impacts of introducing *H. axyridis*, and instructors should encourage discussion and connect any other ecological principle that may have been discussed in the course already (e.g. direct and indirect effects of predation).

The next two questions (questions 18 and 19) ask students to assess the relevance and limitations of this study. Instructors may wish to facilitate a class-wide discussion on the responses here, and highlight the importance of conducting field-based experiments to corroborate results from laboratory

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experiments. Responses may vary to these questions, and students may point out that the study does not provide information on the actual densities of either D. plexippus or H. axyridis in natural populations, nor how likely H. axyridis is to prey on *D. plexippus* eggs or larvae if there are aphids and other prey available. Question 20 then asks students to think of other information or experiments they would want to fully investigate the non-target impacts of H. axyridis on D. plexippus; students may propose experiments where they introduce D. plexippus and aphids as potential prey in varying densities, or design predator choice experiments to test *H. axyridis* predation preferences. In the final question, students return to the original experiment they had designed in part I and reflect on their own experimental design by comparing it to the study's experiments. These questions should allow students the opportunity to strengthen their ability to design well-controlled experiments. Instructors may want to discuss these questions and ask students what they put as strengths and weaknesses of their own experiments, and use this discussion to reinforce lessons on experimental design.

The post-class assessment consists of three questions that may be assigned for homework. The first question asks students to write a short statement to summarize their findings, allowing them to synthesize this information and practice their scientific communication skills. The second question tests student understanding of non-target impacts by asking students to find other potential non-target impacts of *H. axyridis*. The final question assesses student understanding of functional responses and Type I and Type II curves, and also asks students to research Type III curves. Instructors may wish to briefly cover Type III curves and emphasize the differences between these functional responses in a future lesson.

RESOURCES

- Link to the study used in this Figure Set: Koch, R.L., W.D. Hutchison, R.C. Venette, and G.E. Heimpel. 2003. Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biological Control* 28: 265-270. <u>http://www.sciencedirect.com/science/article/pii/S1049964403001026</u>
- Review paper of functional impacts on non-target species of *H. axyridis*: Koch, R.L. 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non-target impacts. *J Insect Sci* 3: 32. <u>https://academic.oup.com/jinsectscience/article/doi/10.1093/jis/3.1.32/854</u> 074/The-multicolored-Asian-lady-beetle-Harmonia

- Suggested papers that involve biological control agents and/or functional responses:
 - Batzli, G.O., Jung, H.G., and G. Guntenspergen. 1981. Nutritional ecology of microtine rodents: linear-foraging rate curves for brown lemmings. Oikos 37: 112-116. http://www.jstor.org/stable/3544080
 - o Buckel, J.A. and A. W. Stoner. 2000. Functional response and switching behavior of young-of-the-year piscivorous bluefish. J Exp Marine Bio and Ecol. 245: 25-41. http://www.sciencedirect.com/science/article/pii/S00220981990015 50#BIB23
 - Claver, M.A., B. Ravichandran, M.M. Khan, and D.P. Ambrose. 0 2003. Impact of cypermethrin on the functional response, predatory and mating behavior of a non-target potential biological control agent Acanthaspis pedestris (stal) (Heet., Reduviidae). J Applied Entomology 127: 18-22. http://onlinelibrary.wiley.com/doi/10.1046/j.1439-0418.2003.00654.x/full
 - o Hu, Y.S., Z.M. Wang, C.L. Ning, Z.Q. Pi, and G.Q. Gao. 1989. The functional response of Harmonia (Leis) axyridis to their prey of Cinara sp. Nat. Enemeis Insects 11: 164-168. https://www.cabdirect.org/cabdirect/abstract/19931168062
 - o Lee, J. and T. Kang. 2004. Functional response of Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) to Aphis gossypii Glover (Homoptera: Aphididae) in the laboratory. *BioControl* 31: 306-310.

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 Řezáč, M., S. Pekár, and J. Stará. 2010. The negative effect of some selective insecticides on the functional response of a potential biological control agent, the spider Philodromus cespitum. BioControl 55: 503-510.

https://link.springer.com/article/10.1007/s10526-010-9272-3

- o Sarmento, R.A., A. Pallini, M. Venzon, O. de Souza, A. Molina-Rugama, and C. de Oliveira. 2007. Functional response of the predator Eriopis connexa (Coleoptera: Coccinellidae) to different prey types. Braz. Archives Biol and Tech. 50: doi10.1590. http://www.scielo.br/scielo.php?script=sci arttext&pid=S1516-89132007000100014
- o Sharma, P.L., S.C. Verma, R.S. Chandel, M.A. Shah, and O. Gavkare. 2017. Functional response of Harmonia dimidiate (fab.) to melon aphid, Aphis gossypii Glover under laboratory conditions.

Phytoparasitica 45: 373-379. https://link.springer.com/article/10.1007/s12600-017-0599-5

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