

LP4 TEACHING PLAN

Reduction and Refinement

BioLEAP
BIOLOGY EDUCATION ADVANCEMENT PROGRAM





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LEARNING PLAN OVERVIEW

In this learning plan, you will be given a more in-depth understanding of the principles of reduction and refinement. You will learn how to use statistical calculations to determine the optimal number of animals that should be used in a study and what husbandry and housing practices can be implemented to ensure animals experience the best possible welfare in a lab environment.

ESTIMATED TOTAL TIME

100 Minutes

PRE-REQUISITE SKILLS

Learning Plan #1 Introduction to Animal Use in Science
Learning Plan #2 The 3Rs of Scientific Research

WHAT STUDENTS WILL LEARN

Competency:

- Apply the principles of reduction and refinement to animal testing

Learning Objectives:

- Define the 3Rs Principles of reduction and refinement
- Describe the benefits of reducing the number of animals used in research
- Identify the importance of sample size selection when applying the principle of reduction
- Apply the resource equation to experimental sample size calculations
- Summarize examples of refinement in experimentation
- Describe how the principle of refinement can be applied to make experiments more humane in cases where animal use cannot be avoided

Assessment:

Monkey House Project Case Study

- Apply reduction and refinement to an animal-based drug study design
- Calculate an optimal sample size for the case study design using the resource equation
- Identify ways to refine the experimental design to enhance animal welfare

Linked External Standards:

NGSS

- HS-ETS1-1 Analyze a major global challenge to specify qualitative and quantitative criteria and constraints for solutions that account for societal needs and wants
- HS-ETS1-3 Evaluate a solution to a complex real-world problem based on prioritized criteria and trade-offs that account for a range of constraints, including cost, safety, reliability, and aesthetics as well as possible social, cultural, and environmental impacts

CCSS- ELA

- RST.11-12.7 Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem

CCSS- Math

- HSS.IC.A.1 Understand statistics as a process for making inferences about population parameters based on a random sample from that population
- HSA.CED.A.3 Represent constraints by equations or inequalities, and by systems of equations and/or inequalities, and interpret solutions as viable or nonviable options in a modeling context

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TEACHING PLAN

#	Learning Activities	Teaching Notes	Materials and Supplies
1	LEARNING PLAN OVERVIEW Review information detailed in the Student Learning Plan.	TIME: 5 minutes ACTIVITY NOTES Provide a brief introduction to the learning plan. This learning plan is designed to follow LP2 in the sequence, The 3Rs of Scientific Research, or a similar introduction to the 3Rs.	Student Learning Plan key words: reduction, refinement, control, sample, sample size, resource equation
2	MOTIVATION ACTIVITY Pretend you are a scientist working in a lab that uses mice. Knowing you are going to use these mice in an experiment, what are some ways that you could improve their life and experience at the lab? Think about which of the 3Rs is reflected by these efforts: Replacement, Reduction, or Refinement?	TIME: 5 minutes ACTIVITY NOTES Ask students to imagine that they are working in a lab that uses mice. Assuming that the animals cannot be replaced, ask students to brainstorm a list of ideas about what welfare improvements they could make to their mouse experiments. Create a list together. Students are encouraged to come up with any ideas regardless of scale or cost.	whiteboard
3	COMPREHENSION ACTIVITY View a presentation on Reduction (one of the 3Rs) and sample sizes.	TIME: 20 minutes ACTIVITY NOTES This presentation gives students more in-depth knowledge about sample size and how the principle of reduction can be applied in experiments by using statistical calculations to determine the minimum number of animals needed for a study. Discuss how experimental numbers can be plugged into the equation and what the results mean.	LP4_3_ReductionAndSampleSize
4	PRACTICE ACTIVITY Explore ways of determining experimental sample sizes using the resource equation. Complete the Applying the Resource Equation worksheet.	TIME: 15 minutes ACTIVITY NOTES Students must use the resource equation to calculate if the proposed sample sizes in animal studies are too large, too small, or optimal. They then must describe how the study is in accordance with or in violation of the principle of reduction.	LP4_4_ ResourceEquationWorksheet LP4_4_ ResourceEquation_AnswerKey

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#	Learning Activities	Teaching Notes	Materials and Supplies
5	COMPREHENSION ACTIVITY Explore ways to manage animal pain, improve housing conditions, and training and handling techniques that reduce stress by viewing the Refinement and Animal Welfare presentation.	TIME: 20 minutes ACTIVITY NOTES This presentation gives students more in-depth knowledge of the principle of refinement and how it can be used to improve animal welfare in the lab. Students will learn about how to manage animal pain, improve housing conditions, and training and handling techniques that reduce stress.	LP4_5_ RefinementandAnimalWelfare
6	PRACTICE ACTIVITY Read the comic, "Rat Park," then complete the Rat Park worksheet. In these activities, you will explore the impact refinement can have on experiments.	TIME: 15 minutes ACTIVITY NOTES Students will read a comic about a famous addiction experiment and answer questions about its implications when considering the principle of refinement.	https://www.stuartmcmillen.com/comic/rat-park/#page-1 LP4_6_RatPark_Worksheet LP4_6_RatPark_AnswerKey
7	APPLICATION ACTIVITY Complete the case study: Monkey House Project worksheet to apply reduction and refinement to a case study. In this assessment, you will be calculating an optimal sample size for the case study design using the resource equation. You will also identify ways to refine the experimental design to enhance animal welfare.	TIME: 20 minutes ACTIVITY NOTES In this case study, students are presented with a scenario where they have been asked to design the housing for marmosets to be used in a drug study. Provide the information with details about the researcher's desires for the study design, marmoset behavior, and welfare recommendations made in the *Guide for Care and Use of Laboratory Animals*. Using this information, students will determine the number of cages and total number of animals to be used based on the principle of reduction, as well as the design elements for each of the cages based on the principle of refinement. Have students complete this individually or in small groups. Discuss the answers.	LP4_7_MonkeyHouse_CaseStudy LP4_7_MonkeyHouse_CaseStudy_ AnswerKey

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Applying the Resource Equation

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Name(s):_____

Date:_____

Read the following research plan proposals and use the resource equation to determine if the proposed sample size is too large, too small, or optimal. Show your work. If you determine the sample size is too large or too small, explain the drawbacks of going forward with the study as proposed and how it violates the principle of reduction.

E = Total number of animals used – Total number of groups
When E > 20, then the sample size is too large
When E < 10, then the sample size is too small

1. A neuroscientist wants to use mice to study the different effects of caffeine on the brain. She decides that she will have one control group and three treatment groups that will receive different doses of caffeine. Each group will have a sample size of 20.



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Applying the Resource Equation

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Name(s):_____

Date:_____

2. A cosmetics company wants to develop a new sunscreen. The product developers need to determine how much of the active ingredient oxybenzone they should include in their formula. They decide to apply sunscreen with varying levels of oxybenzone to seven different groups of two rabbits each and record their level of burns when exposed to sun. They also plan to expose two rabbits to sunlight with no sunscreen at all.

3. The Environmental Protection Agency (EPA) is trying to determine what effects a household cleaner might have on local wildlife if it leaks into the water supply. Researchers distribute 18 fish among 3 tanks filled with untainted pond water and observe their reactions for a week, then the researchers pour a different concentration of the cleaning solution into each tank and observe the fish for another week.



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Applying the Resource Equation

Answer Key

Read the following research plan proposals and use the resource equation to determine if the proposed sample size is too large, too small, or optimal. Show your work. If you determine the sample size is too large or too small, explain the drawbacks of going forward with the study as proposed and how it violates the principle of reduction.

E = Total number of animals used – Total number of groups
When E > 20, then the sample size is too large
When E < 10, then the sample size is too small

1. A neuroscientist wants to use mice to study the different effects of caffeine on the brain. She decides that she will have one control group and three treatment groups that will receive different doses of caffeine. Each group will have a sample size of 20.
   - **Total number of groups = treatment groups + control group = 3 + 1 = 4**
   - **Total number of animals used = sample size x total number of groups = 20 x 4 = 80**
   - **E = Total number of animals used – Total number of groups = 80 – 4 = 76**
   - **76 > 20, therefore the sample size is too large**
   - **Using too many animals increases the amount of time and money dedicated towards an experiment and it unnecessarily wastes lives when fewer animals could have been used to get the same results, violating the principle of reduction.**



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Applying the Resource Equation

Answer Key

2. A cosmetics company wants to develop a new sunscreen. The product developers need to determine how much of the active ingredient oxybenzone they should include in their formula. They decide to apply sunscreen with varying levels of oxybenzone to seven different groups of two rabbits each and record their level of burns when exposed to sun. They also plan to expose two rabbits to sunlight with no sunscreen at all.
   - **Total number of groups = treatment groups + control group = 7 + 1 = 8**
   - **Total number of animals used = sample size x total number of groups = 2 x 8 = 16**
   - **E = Total number of animals used – Total number of groups = 16 – 8 = 8**
   - **8< 10, therefore the sample size is too small**
   - **If the sample size is too small, there is a greater chance the statistical calculations at the end of the study may show no significant difference between the study groups, even though a significant difference between groups does exist in the real population, or vice versa. Animal lives are wasted on an experiment where poor information is attained, and it is likely that the study will have to start over from scratch, requiring even more animals, thus violating the principle of reduction.**

3. The Environmental Protection Agency (EPA) is trying to determine what effects a household cleaner might have on local wildlife if it leaks into the water supply. Researchers distribute 18 fish among 3 tanks filled with untainted pond water and observe their reactions for a week, then the researchers pour a different concentration of the cleaning solution into each tank and observe the fish for another week.
   - **Total number of groups = treatment groups + control group =3 + 3 = 6**
   - **Total number of animals used = 18**
   - **E = Total number of animals used – Total number of groups = 18 – 6 = 12**
   - **10 < 12 < 20, therefore the sample size is optimal**



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The Rat Park Experiment

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Name(s): _____

Date: _____

Read the comic "Rat Park" by Stuart McMillen at www.stuartmcmillen.com/comic/rat-park/#page-1 and answer the following questions.

1. Summarize the results of the experiment. Which rats were more likely to form an addiction: the rats living in isolated cages or those living in the Rat Park? Which rats were more likely to break their addiction?

2. Which enclosure design more closely follows the principle of refinement? What welfare needs are being considered in the design of this enclosure?

3. Consider the original addiction study conducted by scientists in the 1950s and 60s. What was the design flaw in their experiment that led them to believe the availability of drugs would lead to mass addiction in the general population?



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The Rat Park Experiment

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Name(s):_____

Date:_____

4. Which group of rats do you think more closely models the behaviors of the average human adult: those in the Rat Park or those in isolation? Why?

5. Explain how the results of this study can be used to support the claim that improving the well-being of lab animals can make them better experimental models for the general population.



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The Rat Park Experiment

Answer Key

Read the comic "Rat Park" by Stuart McMillen at www.stuartmcmillen.com/comic/rat-park/#page-1 and answer the following questions.

1. Summarize the results of the experiment. Which rats were more likely to form an addiction: the rats living in isolated cages or those living in the Rat Park? Which rats were more likely to break their addiction?
   The rats living in isolated cages were more likely to become addicted to morphine, consuming 19 times more morphine than their counterparts in the Rat Park. When all the rats were purposely conditioned with a morphine addiction, those living in the rat park would try to avoid being drugged when given the choice, while those in isolation maintained their drug habit.

2. Which enclosure design more closely follows the principle of refinement? What welfare needs are being considered in the design of this enclosure?
   The design of the Rat Park enclosure more closely follows the principle of refinement, which states that animal distress should be minimized in experimentation. The Rat Park was 200 times the size of a regular cage, giving rats room to move around. It included wheels to run on, wood shavings to nest in, and boxes to hide in, all of which allowed the rats to perform natural behaviors. Most importantly, the rats were able to socialize with members of their own species.

3. Consider the original addiction study conducted by scientists in the 1950s and 60s. What was the design flaw in their experiment that led them to believe the availability of drugs would lead to mass addiction in the general population?
   The rats in the original study were kept in isolated, bare cages that in no way resembled their natural habitat. The lack of mental or social stimulation led rats to form severe drug addictions across the board, leading researchers to conclude that if drugs were made available to the general human population, there would be an addiction epidemic.



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The Rat Park Experiment

Answer Key

4. Which group of rats do you think more closely models the behaviors of the average human adult: those in the Rat Park or those in isolation? Why?
   Those in the Rat Park. Much like the average human adult, these rats had the freedom to move around, pursue their natural behaviors, and socialize with members of their species. A rat in an isolated cage might model the behavior of a human incarcerated in solitary confinement or, as the comic suggests, a socially isolated person, but not the average adult.

5. Explain how the results of this study can be used to support the claim that improving the well-being of lab animals can make them better experimental models for the general population.
   This experiment demonstrates how the extreme psychological duress experienced by rats in isolated cages skews the results by adding an extra variable to the study. The abnormal condition of their environment leads to abnormal behavior and, thus, useless results. Animals who are kept in good welfare conditions that resemble the freedoms that the average human being has will exhibit behaviors that more closely model human reactions.



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Welcome to the Monkey House

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Name(s):_____

Date:_____

You've joined a research team that is planning to use the common marmoset in a study testing the effect of different dosages of a new treatment for multiple sclerosis, an autoimmune disease that affects the central nervous system. Your team would like to test 3 different dosages of the drug and observe a separate group of marmosets that will receive zero doses of the drug. Based on your knowledge of the principle of reduction, help the team determine how many marmosets to use in the study.

E = Total number of animals used – Total number of groups
When E > 20, then the sample size is too large
When E < 10, then the sample size is too small

About the Common Marmoset

The common marmoset (*Callithrix jacchus*) is species of monkey that is favored for use in research because of its small size; it weighs just 230-260 grams at maturity. This tiny primate originates from Brazil, where it can be found living in forests and cities. They are a territorial species and live in family groups of 4 to 15 individuals made up of one breeding female, one breeding male, and their offspring. Marmosets spend most of their time in trees, foraging in the canopy 2-5 meters above the ground. They are omnivores, eating a variety of fruits and insects, but most of their diet is made up of sap that they extract from trees by gnawing through the bark.

Valença-Montenegro, M.M., Bezerra, B.M., Ruiz-Miranda, C.R., Pereira, D.G., Miranda, J.M.D., Bicca-Marques, J.C., Oliveira, L., da Cruz, M.A.O.M., Valle, R.R. & Mittermeier, R.A. 2021. Callithrix jacchus (amended version of 2018 assessment). The IUCN Red List of Threatened Species



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Welcome to the Monkey House

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RECOMMENDED MINIMUM SPACE FOR NONHUMAN PRIMATES HOUSED IN PAIRS OR GROUPS

Animals	Weight,[a] kg	Floor area/animal,[b] ft² (m²)	Height,[c] in (cm)	Comments
Monkeys[d] (including baboons)				Cage height should be sufficient for the animals to comfortably stand erect with their feet on the floor. Baboons, patas monkeys, and other longer-legged species may require more height than other monkeys, as might long-tailed animals and animals with prehensile tails. Overall cage volume and linear perch space should be considerations for many neotropical and arboreal species. For brachiating species cage height should be such that an animal can, when fully extended, swing from the cage ceiling without having its feet touch the floor. Cage design should enhance brachiating movement.
Group 1	Up to 1.5	2.1 (0.2)	30 (76.2)	
Group 2	Up to 3	3.0 (0.28)	30 (76.2)	
Group 3	Up to 10	4.3 (0.4)	30 (76.2)	
Group 4	Up to 15	6.0 (0.56)	32 (81.3)	
Group 5	Up to 20	8.0 (0.74)	36 (91.4)	
Group 6	Up to 25	10 (0.93)	46 (116.8)	
Group 7	Up to 30	15 (1.4)	46 (116.8)	
Group 8	>30[e]	≥25 (2.32)	60 (152.4)	
Animals	**Weight,[a] kg**	**Floor area/animal,[b] ft² (m²)**	**Height,[c] in (cm)**	**Comments**
Chimpanzees (PAN)				For other apes and large brachiating species cage height should be such that an animal can, when fully extended, swing from the cage ceiling without having its feet touch the floor. Cage design should enhance brachiating movement.
Juveniles	Up to 10	15 (1.4)	60 (152.4)	
Adults[f]	>10	≥25 (2.32)	84 (213.4)	



[a]To convert kilograms to pounds, multiply by 2.2.

[b]Singly housed primates may require more space than the amount allocated per animal when group housed.

[c]From cage floor to cage top.

[d]Callitrichidae, Cebidae, Cercopithecidae, and Papio.

[e]Larger animals may require more space to meet performance standards (see text).

[f]Apes weighing over 50 kg are more effectively housed in permanent housing of masonry, concrete, and wire-panel structure than in conventional caging.

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011.

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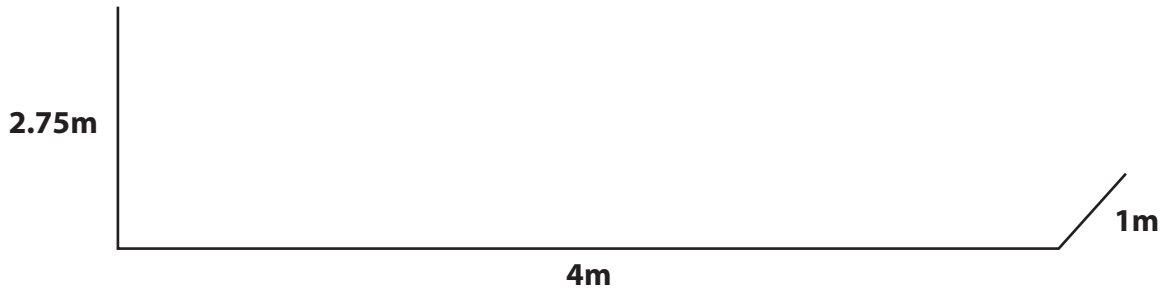
Welcome to the Monkey House

Next, your team needs to figure out how to house the research subjects. There is a limited amount of space where the cages can be kept in the lab, and the team needs to determine how many animals can comfortably fit per cage and how best to design the cages. The space the research team has allotted for cages is 2.75 meters tall, 1 meter wide, and 4 meters long. Based on provided information about common marmoset behavior, caging standards set by the *Guide for the Care and Use of Laboratory Animals*, and your knowledge of applying the principles of refinement, help your team answer the following questions.

1. How many cages should be built?

2. How many marmosets will live in each cage?

3. What will the dimensions of the cages be? Sketch what they would look like in the given space Assume there is no need to account for space between cages and that cages may be stacked on top of each other if necessary.



4. What three additional features would you add to the cages to improve welfare conditions for the marmosets?



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Welcome to the Monkey House

Answer Key

You've joined a research team that is planning to use the common marmoset in a study testing the effect of different dosages of a new treatment for multiple sclerosis, an autoimmune disease that affects the central nervous system. Your team would like to test 3 different dosages of the drug and observe a separate group of marmosets that will receive zero doses of the drug. Based on your knowledge of the principle of reduction, help the team determine how many marmosets to use in the study.

E = Total number of animals used – Total number of groups
When E > 20, then the sample size is too large
When E < 10, then the sample size is too small

10 < total number of marmosets used – total number of groups < 20
10 < total number of marmosets used – 4 < 20
14 < total number of marmosets used < 24

Because there are 4 groups and each group must have the same sample size, you know that the total number of marmosets used must be divisible by 4.

Numbers between 14 and 24 that are divisible by 4: 16, 20

The principle of reduction says that the fewest number of animals should be used, so the optimal number of marmosets to use in this study is 16.



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Welcome to the Monkey House

Answer Key

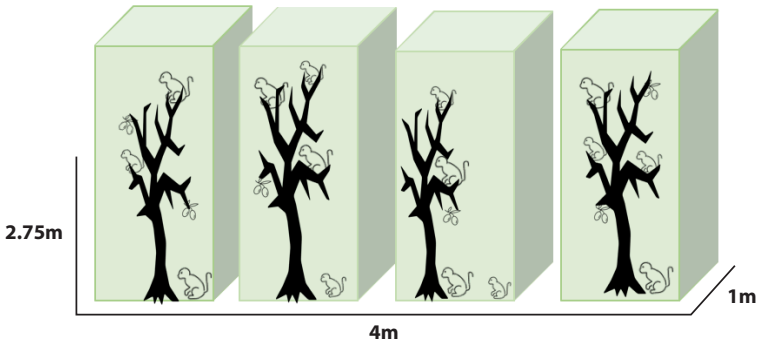
Next, your team needs to figure out how to house the research subjects. There is a limited amount of space where the cages can be kept in the lab, and the team needs to determine how many animals can comfortably fit per cage and how best to design the cages. The space the research team has allotted for cages is 2.75 meters tall, 1 meter wide, and 4 meters long. Based on provided information about common marmoset behavior, caging standards set by the *Guide for the Care and Use of Laboratory Animals*, and your knowledge of applying the principles of refinement, help your team answer the following questions.

1. How many cages should be built?
   In this scenario, it would be appropriate to build four different cages, one for each of the three treatment groups and one for the control group. While building more cages of a smaller size is possible, building fewer cages of a larger size will allow the marmosets to be housed in larger social groups, which will improve their welfare.

2. How many marmosets will live in each cage?
   Each cage would hold 4 marmosets, ideally a breeding pair and their offspring to mimic natural family size and structure. However, certain experimental conditions may require that the monkeys all be the same sex and age, so this exact social structure is not always possible.

3. What will the dimensions of the cages be? Sketch what they would look like in the given space Assume there is no need to account for space between cages and that cages may be stacked on top of each other if necessary.
   Marmosets weigh less than 1.5 kilograms, so at minimum they need 0.2m^2 of floor space per animal, meaning a cage of 4 marmosets must have 0.8m^2 of floor space. While both building the cages side by side and stacked on top of each other in two rows would meet the minimum floor space and height requirements, students should note the importance of vertical space for tree dwelling primates and choose the side-by-side option giving each cage 1m^2 of floor space and 2.75 meters of vertical height.





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Welcome to the Monkey House

Answer Key

4. What three additional features would you add to the cages to improve welfare conditions for the marmosets?
   Suggestions for other welfare improvements may include constant access to clean fresh water and nutritious food, tree-like structures for climbing, food hidden in puzzle toys high in the cage to promote foraging behavior, and bark that the marmosets can chew to extract sap.

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Student correctly calculates that 14 < total number of marmosets < 24 (1 pt).

Student reasons that the ideal number of marmosets to use in the study is 16 (1 pt).

Student correctly identifies that the marmosets should be housed in groups (1 pt) of 4 (1 pt).

Student designs 4 cages (1 pt) built to take advantage of all 2.75 meters of vertical space (1 pt).

Student adds three additional enrichment features like trees, food puzzles, chewing bark (3 pts).



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