The Competitive Exclusion Principle, or Gause's law, proposes that two species competing for the same limited resources cannot sustainably coexist or maintain constant population values. Intraspecific competition, describes when organisms within the same species compete for resources; leading the population to reach carrying capacity. Carrying capacity refers to the maximum population size a species can sustain within its environmental limitations. Interspecific competition describes when competition for resources occurs between different species of organisms. Species can be limited by both their carrying capacity (intraspecific competition) and the interspecific competition. When two species compete within the same ecological niches, the Competitive Exclusion Principle predicts that the better adapted species, even if only slightly better adapted, will drive the other to local extinction. In the 1930s, biologist Georgy Gause explored the idea of interspecific competition in a groundbreaking study of competition in Paramecium. Paramecia are aquatic single-celled Ciliates that survive on a diet of Bacteria, Yeast, Algae, and other small protozoa. Based on the findings of this experiment and other research, Gause developed the Competitive Exclusion Principle.

In this investigation, students explore how competition affects population growth, and put the Competitive Exclusion Principle to the test. Following a very similar experiment design to Gause's original Paramecium experiment, students examine two Paramecium species; Paramecium Caudatum and Paramecium Aurelia. These two species make great model organisms to test this principle due to their similarity, and the fact they compete directly for food. However, the two vary in size with Paramecium Caudatum approximately four times the size of Paramecium Aurelia. Students are tasked with growing the two species both separately and together in a culture medium. The three cultures samples are maintained within the exact same environmental conditions, as Gause's law only applies if the ecological factors are constant. Over three weeks, students observe population growth to determine how competition for resources affects population growth.

### Materials

- Pure cultures of Paramecium Caudatum
- Pure cultures of Paramecium Aurelia
- Paramecium Culture Medium
- 3 Deep Petri Dishes
- Sedgewick Rafter Cell
- Graduated Cylinder
- Mosquito Net/Cheese Cloth
- Plastic Pipettes
- Compound Microscope

### Safety Precautions

- Wear appropriate Personal Protective Equipment (PPE), including gloves and lab coat.
- Paramecia are harmless to humans, but swamp or pond water may contain pathogens. Wash hands thoroughly before and after handling living specimens.

### Preparation - By Lab Technician

#### Preparing Cultures

1. As soon as your shipment arrives, open the shipping container, remove the Paramecium culture jars, and inspect your culture.
2. Loosen the lids on the jars and aerate the culture using the supplied plastic pipette.
3. To bubble air into the water, hold the pipette tip into the culture water and squeeze the bulb. Raise the pipette; releasing the bulb; allowing it to fill with air once again.
4. Repeat this step four more times to assist in replacing the oxygen depleted during shipping.
• State your hypothesis.

• Label three clean petri dishes as follows.

• Add 50mL of Paramecium culture medium to each dish.

• Using a graduated cylinder, transfer 20 mL of Paramecium Aurelia into “Petri Dish A”.

• Transfer 20 mL of Paramecium Caudatum and 20 mL of Paramecium Aurelia into “Petri Dish B”.

• Transfer 20 mL of Paramecium Caudatum into “Petri Dish C”.

• Add six grains of rice to the dish to sustain the paramecium and cover each of the petri dishes containing the Paramecium cultures with cheesecloth.

• Store the petri dishes at a consistent temperature of 24ºC on a flat surface where they will not be disturbed. Keep away from direct sunlight.

• Using a fresh sterile pipette, place 1mL of liquid from each “Petri Dish A” into a Sedgewick Rafter cell.

• Using a compound microscope, count the relative number of Paramecium. If the population becomes too dense to count, choose one transect (line of 20 squares representing 20 µL) and multiply by 50 to get the population density per mL.

• Return specimens to the Petri dish. This reduces population loss as a result of the counting procedure.

• Thoroughly wash the Sedgewick Rafter cell to fully remove all Paramecium specimens.

• Repeat steps 9-12 for “Petri Dish B & C”. For “Petri Dish B”, count the two species separately. The Paramecium species can be differentiated by their size. Paramecium Caudatum is approximately four times the size of Paramecium Aurelia.

• Record your data in a table.

• Repeat counting procedure every second day for three weeks and record the results.

Preparing workstations

1. Provide each workstation with:
   - Pure cultures of Paramecium Caudatum
   - Pure cultures of Paramecium Aurelia
   - Paramecium Culture Medium
   - 3 Deep Petri Dishes
   - Sedgewick Rafter Cell
   - Graduated Cylinder
   - Mosquito Net/CheeseCloth
   - Sterile Plastic Pipettes
   - Compound Microscope

METHOD - STUDENT PRACTICAL

1. State your hypothesis.

2. Label three clean petri dishes as follows.

   A
   Paramecium Aurelia

   B
   Both

   C
   Paramecium Caudatum

3. Add 50mL of Paramecium culture medium to each dish.

4. Using a graduated cylinder, transfer 20 mL of Paramecium Aurelia into “Petri Dish A”.

5. Transfer 20 mL of Paramecium Caudatum and 20 mL of Paramecium Aurelia into “Petri Dish B”.

6. Transfer 20 mL of Paramecium Caudatum into “Petri Dish C”.

7. Add six grains of rice to the dish to sustain the paramecium and cover each of the petri dishes containing the Paramecium cultures with cheesecloth.

8. Store the petri dishes at a consistent temperature of 24ºC on a flat surface where they will not be disturbed. Keep away from direct sunlight.

9. Using a fresh sterile pipette, place 1mL of liquid from each “Petri Dish A” into a Sedgewick Rafter cell.

10. Using a compound microscope, count the relative number of Paramecium. If the population becomes too dense to count, choose one transect (line of 20 squares representing 20 µL) and multiply by 50 to get the population density per mL.

11. Return specimens to the Petri dish. This reduces population loss as a result of the counting procedure.

12. Thoroughly wash the Sedgewick Rafter cell to fully remove all Paramecium specimens.

13. Repeat steps 9-12 for “Petri Dish B & C”. For “Petri Dish B”, count the two species separately. The Paramecium species can be differentiated by their size. Paramecium Caudatum is approximately four times the size of Paramecium Aurelia.

14. Record your data in a table.

15. Repeat counting procedure every second day for three weeks and record the results.
**OBSERVATION AND RESULTS**

Below is an example of expected results based on initial population density of Paramecium were 10 per 1 mL. This is to be used as a guide only as individual results will vary.

<table>
<thead>
<tr>
<th>Days (3 weeks)</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
<th>13</th>
<th>15</th>
<th>17</th>
<th>19</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separate cultures (Petri Dishes A&amp;C) Paramecium Aurelia</td>
<td>10</td>
<td>100</td>
<td>230</td>
<td>280</td>
<td>290</td>
<td>250</td>
<td>260</td>
<td>220</td>
<td>250</td>
<td>220</td>
<td>250</td>
</tr>
<tr>
<td>Paramecium Caudatum</td>
<td>10</td>
<td>150</td>
<td>530</td>
<td>740</td>
<td>900</td>
<td>840</td>
<td>980</td>
<td>800</td>
<td>940</td>
<td>970</td>
<td>980</td>
</tr>
<tr>
<td>Combined cultures (Petri Dish B) Paramecium Aurelia</td>
<td>10</td>
<td>70</td>
<td>80</td>
<td>80</td>
<td>70</td>
<td>60</td>
<td>30</td>
<td>15</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Paramecium Caudatum</td>
<td>10</td>
<td>140</td>
<td>330</td>
<td>520</td>
<td>540</td>
<td>600</td>
<td>640</td>
<td>720</td>
<td>600</td>
<td>580</td>
<td>540</td>
</tr>
</tbody>
</table>

*Table 1: Expected results*

**INVESTIGATIONS**

- Ask students whether their hypothesis was proven correct. Students should provide an explanation as to why/why not.
- Ask students to describe the advantages and disadvantages of this counting technique.
- Challenge students to describe limitations in the experiment design. Ask them to suggest ways it can be improved.
- Compare the population sizes of each Paramecium species in the separated (A&C) and mixed cultures (B). Ask students what the results reveal about how competition affects population growth.
- Ask students to explain whether results prove or disprove the Competitive Exclusion Principle.

**EXTENSION EXERCISE**

- Exponential growth describes population growth that is unlimited. Logistic growth describes growth rates that are limited by a number of factors; including, predators, food scarcity, as well as competition for food and habitat. Which type of growth was exhibited in the Paramecium populations containing only one of the species?

**TEACHING NOTES**

- Demonstrate the correct method of preparing the Paramecium cultures in the petri dish before students begin the procedure.
- Students may be tempted to add too much rice to each petri dish; however, inform students that 6 grains of uncooked rice will be sufficient.
- Ensure students understand the importance of following the exact same procedure for each culture, to generate the most accurate results possible. Instruct students to add the exact same measurement of cultures to each petri dish; add the same number of rice grains; use the same method for counting; and maintain the culture within the same environmental conditions. Environmental conditions include access to light and temperature.
- There are quieting solutions, such as Protoslo®, available that will allow you to slow the organism’s movement without damaging them. This makes it easier for students to count the Paramecium culture under the microscope.