Species that have specialized resource or habitat requirements may be under particular risk if their required resources are threatened. Populations of the coastal horned lizard have recently declined in response to loss and fragmentation of their coastal habitats in central California. These population declines could be driven by bottom-up changes to their food supply that have resulted from displacement of native ants (*Crematogaster californica*) by introduced Argentine ants (*Linepithema humile*), by top-down effects of predatory domesticated cats that can infiltrate their habitats along edges that are close to human habitation, or by other effects of habitat alteration. The purpose of this study will be to determine whether bottom-up effects of changes in the lizards’ food supply are sufficient to explain declines in their populations, or whether potential food resources that remain after invasion by Argentine ants can sustain their populations, signaling that a different problem is at work.

II. Questions and Predictions

A. Research question(s): Are lizards able to make use of foods other than the native ants that they specialize on, which have declined, namely Argentine ants or other non-ant arthropods that have persisted in their habitats?

B. Critical prediction: If lizards can maintain positive growth rates only when fed native ants, but not when fed introduced ants or non-ant arthropods…and if the reason they cannot persist on non-ant arthropods is that they are too difficult to capture…and if I provide them in the laboratory with these three different resources, as well as a non-ant arthropod that is easy to catch…then they will maintain positive growth rates only on the native ants or on non-ant arthropods that are made easy to catch.

III. Methods

A. Main details of experimental design

1. Subjects: 40 individual hatchling coastal horned lizards distributed among 4 treatments

2a. Treatment variables (numbered list of treatments showing factor levels that differ across treatments):

1) Native ants provided as food in lizard enclosures
2) Introduced ants provided as food in lizard enclosures
3) Non-ant arthropods collected from the lizard’s habitat provided as food in lizard enclosures
4) Crickets with one leg removed to facilitate capture provided as food in lizard enclosures

2b. Condition constants (non-variable factors that will be held similar across treatments):
Lizards will housed individually in terraria containing soil, water, and vegetation. Temperature and light conditions will be maintained using heat and light lamps on cycles to simulate day/night temperatures and lighting conditions in their natural habitat (36-40C).

2c. General procedure for applying treatments to subjects: Food for a given treatment will be added daily to maintain an ad libitum supply.
2d. **Response variable(s), including how and when measured:**

Body size will be measured at the start and end of the experimental period of 5 weeks using a balance to measure mass. Changes in mass will be calculated relative to initial mass, and used to compare growth rates across treatments.

### 3a. How treatment comparisons will isolate the effects of experimental variables

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Variable isolated</th>
<th>Prediction about response variable [alternate predictions]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs. 2</td>
<td>Ant species (native vs. introduced)</td>
<td>Growth will be greater when supplied with native species [growth will not differ between ant species, signaling that the introduced ant serves as a food replacement for lizards]</td>
</tr>
<tr>
<td>1 vs. 3</td>
<td>Native food source</td>
<td>Growth will be greater on ants than on other arthropods given specialization [growth will not differ, signaling that residual food sources are sufficient after invasion and elimination of native ant]</td>
</tr>
<tr>
<td>3 vs. 4</td>
<td>Catchability of prey</td>
<td>Immobilization of non-ant arthropods will lead to greater growth rate [growth will not differ between treatments, signaling that non-ant arthropods are insufficient resource because of their nutritional content, not their catchability].</td>
</tr>
</tbody>
</table>

### 3b. How these comparisons together provide a test of the critical prediction; alternative results and how they would be interpreted:

If my prediction is supported, the lizards will only be able to maintain positive growth rates on their specialized prey, and bottom-up effects will be implicated. If it is not supported—either because they can maintain positive growth on introduced ants or on non-ant arthropods—it would suggest that factors other than changes in food supply are responsible for declines in lizard populations.

### B. Other aspects of experimental design

1. **Location, timing, and duration of experiments, any other experimental conditions:** Experiments will take place in terraria in the laboratory, with ants and non-ant arthropods collected from lizard habitats and supplied fresh to terraria each day so that food is not limited. To minimize impact to habitats, commercially ordered crickets will be used in treatment 4.

2. **Bias will be minimized:** The four treatments will be assigned randomly to lizard subjects, and terraria in which lizards are housed will be positioned randomly in the lab with respect to treatment.

3. **How noise will be minimized (include a description of the resource that will limit the size of the experiment—material, space, time, money—over all replicates):** Ten replicates of each treatment will be used. This experiment size is determined both by the need to minimize the number of these endangered animals collected from the wild and the size of the space available in the lab for terraria.

4. **Recognition of any potential ethical issues, and how they will be handled:** This endangered lizard will be collected as hatchlings and will be returned to their natal field locations after experiments. Any lizards that appear to be suffering health consequences from captivity will be released.

### IV. HIGH-ImpACT SUMMARY OF SIGNIFICANCE OF PROPOSED RESEARCH

Future efforts to manage endangered populations of coastal horned lizards will require an understanding of the specific factors that are leading to their decline. The two leading hypotheses for their decline—that they suffer bottom-up effects of the loss of their native food source, or top-down effects of introduced cat predators—have not been fully evaluated. Identifying the cause of the decline will help to focus conservation efforts on general habitat requirements or specific dietary needs.

### Sketch:

Attach a scan (or alternatively, bring to class on Wednesday) a sketch or flowchart that helps to explain your experimental design. Initial here if a hard copy will be handed in → AS