Participants of the Dartmouth Biology FSP 2013

Faculty
Ryan G. Calsbeek  Matthew P. Ayres  Brad W. Taylor

Lab Coordinator
Craig D. Layne

Graduate Assistants
Zachariah J. Gezon  Ramsa Chaves-Ulloa

Undergraduates
Amelia F. Antrim  Emilia H. Hull
Tyler E. Billip  Eliza W. Huntington
Gillian A. O. Britton  Ellen T. Irwin
Seth A. Brown  Kali M. Pruss
Colleen P. Cowdery  Molly R. Pugh
Jimena Diaz  Elisabeth R. Seyferth
Samantha C. Dowdell  Victoria D. H. Stein
Maria Isabel Regina D. Francisco
Dartmouth Studies in Tropical Ecology, Vol. 23

Dartmouth College runs an annual 9-10 week ecological field research program in Costa Rica and the Caribbean. Manuscripts from the research projects in this program have been published in the annual volume “Dartmouth Studies in Tropical Ecology” since 1989. Copies are held in the Dartmouth library and in Costa Rica at the San Jose office of the Organization for Tropical Studies (OTS/OET), at the OTS field stations at Palo Verde, Las Cruces and La Selva, at the Cuerici Biological Station, at the Sirena Station of the Corcovado National Park, and at the Monteverde Biological Station. On Little Cayman Island, there are copies at the Little Cayman Research Center.

Dartmouth faculty from the Department of Biological Sciences, along with two Ph.D. students from Dartmouth’s Ecology and Evolutionary Biology graduate program, advise ca. 15 advanced undergraduate students on this program. The first few projects are designed by the advisors, but undergraduates soon begin conceiving and designing their own projects.

The order of authorship on each paper is alphabetical, in keeping with the style of the program, which emphasizes a cooperative and egalitarian relationship among undergraduates in each project. Where faculty or graduate student mentors have pre-designed a project, this is indicated after the author listing at the head of the paper. For each paper there is a faculty editor (also indicated after the author listing), who takes responsibility for defining the required revisions, and decides on the acceptability of manuscripts for publication. On each paper, at least one faculty member and one graduate student are heavily involved as mentors at every stage, from project design to final manuscript. However, it is our policy that faculty and graduate students are not included as authors for undergraduate projects. Our annual books do include a few exceptions, i.e. projects initiated and conducted by graduate students; these tend to be rare, due to the heavy research advising commitments of Ph.D. students on the program.

We thank the Costa Rican Ministry of the Environment and Energy (MINAE) for permission to conduct research in Costa Rica’s extraordinary national parks. The Organization for Tropical Studies (OTS/OET) has provided essential support for our program for over 30 years, taking care of most of our logistical needs in Costa Rica, always to high standards of quality and reliability. We thank OTS staff at the Palo Verde and La Selva Biological Stations, and at the Wilson Botanical Garden at Las Cruces, for all their services rendered efficiently, politely and in good spirit. Staff at the Santa Rosa and Corcovado National Parks have also been gracious in accommodating and assisting us. We thank Carlos Solano at the Cuerici Biological Station for his depth of knowledge and inspiration. We are grateful to the staff of the Monteverde Biological Station for access to their facilities, and for making us so comfortable when we arrive late, dirty, hungry and tired from Santa Rosa.

On Little Cayman Island, the Little Cayman Research Center (LCRC), operated by the Central Caribbean Marine Institute, is our base for the entire coral reef ecology segment of the program. Expert LCRC staff run the lab, provide accommodations and food, operate research vessels and take care of SCUBA diving logistics and safety. On the Dartmouth campus, the Off Campus Programs Office, under the Associate Dean of International and Interdisciplinary Studies, deals with administration and emergency services and provides an essential lifeline to remote locations in rare times of need.

We acknowledge the generous financial support of Dorothy Hobbs Kroenlein.

For further information about this volume or the program in general, contact the Program Director or the Department of Biological Sciences at Dartmouth College, Hanover New Hampshire, USA (http://www.dartmouth.edu/~biology/)

Matthew P. Ayres
Professor of Biological Sciences and Director of Biology Foreign Studies Program
Life Sciences Center, 78 College Street
Dartmouth College
Hanover, NH 03755
603 646-2788 lab
603 359-7231 cellphone
http://www.dartmouth.edu/~mpayres/
Matt.Ayres@Dartmouth.Edu
<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Morning</th>
<th>Afternoon</th>
<th>Evening</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Jan</td>
<td>To San Jose</td>
<td>Travel</td>
<td>Travel</td>
<td>Arrive</td>
</tr>
<tr>
<td>8-Jan</td>
<td>At SJ</td>
<td>OTS, InBio</td>
<td>free</td>
<td>Group dinner</td>
</tr>
<tr>
<td>9-Jan</td>
<td>To Palo Verde</td>
<td>Travel</td>
<td>Orientation</td>
<td>Lec: Intro CR (RCU, ES)</td>
</tr>
<tr>
<td>10-Jan</td>
<td>At PV</td>
<td>Orientation</td>
<td>Lec: Primate Ecol (RC,AA)</td>
<td>Lec: Avian ecol (RC,JD)</td>
</tr>
<tr>
<td>11-Jan</td>
<td>At PV</td>
<td>FP-1</td>
<td>Stat lab (ZG)</td>
<td>Analysis</td>
</tr>
<tr>
<td>12-Jan</td>
<td>At PV</td>
<td>FP-2</td>
<td>Arth lab (RCU), Lec: Behav (RC,MP)</td>
<td>FP-1 symposium. Writing</td>
</tr>
<tr>
<td>13-Jan</td>
<td>At PV</td>
<td>FP-2</td>
<td>Lec: Div/Co-ex (ZG,VS)</td>
<td>Writing. FP-1 ms due</td>
</tr>
<tr>
<td>14-Jan</td>
<td>At PV</td>
<td>SIFP-1 plan</td>
<td>Plant Lab (RC,RCU,ZG), Writing</td>
<td>FP-2 seminar. Writing</td>
</tr>
<tr>
<td>15-Jan</td>
<td>At PV</td>
<td>SIFP-1</td>
<td>SIFP-1</td>
<td>Writing. FP-2 ms due</td>
</tr>
<tr>
<td>16-Jan</td>
<td>At PV</td>
<td>SIFP-1</td>
<td>SIFP-1 analysis, revisions</td>
<td>SIFP-1 analysis, revisions</td>
</tr>
<tr>
<td>17-Jan</td>
<td>At PV</td>
<td>River trip</td>
<td>SIFP-1 symposium. Writing</td>
<td>SIFP-1 ms due</td>
</tr>
<tr>
<td>18-Jan</td>
<td>To Santa Rosa</td>
<td>Travel/walk</td>
<td>Orientation. Lec: Turtles. Vert lab (RC)</td>
<td>Field: Sea turtle nesting</td>
</tr>
<tr>
<td>19-Jan</td>
<td>At SR</td>
<td>Lec:MNGRV(NL)</td>
<td>Exploration</td>
<td>Field: Sea turtle nesting</td>
</tr>
<tr>
<td>20-Jan</td>
<td>To Monteverde</td>
<td>Travel</td>
<td>Orientation</td>
<td>Writing, revisions</td>
</tr>
<tr>
<td>21-Jan</td>
<td>At MV</td>
<td>Orientation</td>
<td>SIFP-2 planning</td>
<td>Lec: Amphibians</td>
</tr>
<tr>
<td>22-Jan</td>
<td>At MV</td>
<td>SIFP-2 pilot</td>
<td>SIFP-2</td>
<td>Lec: His/Orig (RC,CC)</td>
</tr>
<tr>
<td>23-Jan</td>
<td>At MV</td>
<td>SIFP-2</td>
<td>SIFP-2</td>
<td>Analysis, writing</td>
</tr>
<tr>
<td>24-Jan</td>
<td>At MV</td>
<td>SIFP-2</td>
<td>SIFP-2</td>
<td>Analysis, writing</td>
</tr>
<tr>
<td>25-Jan</td>
<td>At MV</td>
<td>Writing</td>
<td>SIFP-2 ms due</td>
<td>Writing</td>
</tr>
<tr>
<td>26-Jan</td>
<td>At MV</td>
<td>Final ms due</td>
<td>Exploration</td>
<td>Free</td>
</tr>
<tr>
<td>27-Jan</td>
<td>To Cuerici</td>
<td>Travel</td>
<td>Travel, orientation</td>
<td>Lec: Coevol (MA,SD)</td>
</tr>
<tr>
<td>28-Jan</td>
<td>At Cuerici</td>
<td>Paramo</td>
<td>Orientation</td>
<td>Writing lab 1 (MA)</td>
</tr>
<tr>
<td>29-Jan</td>
<td>At Cuerici</td>
<td>SIFP-3 planning</td>
<td>SIFP-3 pilot</td>
<td>SIFP-3 proposals</td>
</tr>
<tr>
<td>30-Jan</td>
<td>At Cuerici</td>
<td>SIFP-3</td>
<td>SIFP-3</td>
<td>Lec: Coevol (MA,KP)</td>
</tr>
<tr>
<td>31-Jan</td>
<td>At Cuerici</td>
<td>SIFP-3</td>
<td>SIFP-3</td>
<td>Analysis, writing</td>
</tr>
<tr>
<td>1-Feb</td>
<td>At Cuerici</td>
<td>Analysis, writing</td>
<td>SIFP-3 symposium</td>
<td>SIFP-3 ms due</td>
</tr>
<tr>
<td>2-Feb</td>
<td>To La Palma</td>
<td>Exploration</td>
<td>Travel to La Palma</td>
<td>Sieran prep</td>
</tr>
<tr>
<td>3-Feb</td>
<td>To Sirena</td>
<td>Hike</td>
<td>Hike to Sirena</td>
<td>Natural history reports</td>
</tr>
<tr>
<td>4-Feb</td>
<td>At Sirena</td>
<td>Orientation</td>
<td>SIFP-4 plan</td>
<td>Lec: Social insects (ZG,IF)</td>
</tr>
<tr>
<td>5-Feb</td>
<td>At Sirena</td>
<td>SIFP-4 pilot</td>
<td>SIFP-4</td>
<td>Lec: Plant/Herb intrxn</td>
</tr>
<tr>
<td>6-Feb</td>
<td>At Sirena</td>
<td>SIFP-4</td>
<td>SIFP-4</td>
<td>Writing lab 2 (MA)</td>
</tr>
<tr>
<td>7-Feb</td>
<td>At Sirena</td>
<td>SIFP-4</td>
<td>SIFP-4</td>
<td>Analysis, writing</td>
</tr>
<tr>
<td>8-Feb</td>
<td>At Sirena</td>
<td>SIFP-4</td>
<td>Analysis, writing</td>
<td>SIFP-4 symposium</td>
</tr>
<tr>
<td>9-Feb</td>
<td>To Las Cruces</td>
<td>Hike</td>
<td>Travel</td>
<td>Writing</td>
</tr>
<tr>
<td>10-Feb</td>
<td>At Las Cruces</td>
<td>Orientation</td>
<td>Writing, botany</td>
<td>Writing</td>
</tr>
<tr>
<td>11-Feb</td>
<td>At Las Cruces</td>
<td>Writing, botany</td>
<td>Writing, botany practicum</td>
<td>Discussion: Why science?</td>
</tr>
<tr>
<td>12-Feb</td>
<td>To La Selva</td>
<td>Travel</td>
<td>Travel</td>
<td>Analysis, writing</td>
</tr>
<tr>
<td>13-Feb</td>
<td>At La Selva</td>
<td>Orientation</td>
<td>SIFP-5 plan/pilot</td>
<td>Lec: Aquatic Eco (RCU, TB)</td>
</tr>
<tr>
<td>14-Feb</td>
<td>At La Selva</td>
<td>SIFP-5</td>
<td>SIFP-5</td>
<td>Writing, Night walk</td>
</tr>
<tr>
<td>15-Feb</td>
<td>At La Selva</td>
<td>SIFP-5</td>
<td>Agroecology field trip</td>
<td>Lec: Ecosystems (MA,EH)</td>
</tr>
<tr>
<td>16-Feb</td>
<td>At La Selva</td>
<td>SIFP-5</td>
<td>SIFP-5</td>
<td>Paper (ED), writing, night walk</td>
</tr>
<tr>
<td>17-Feb</td>
<td>At La Selva</td>
<td>SIFP-5</td>
<td>Analysis, writing</td>
<td>Paper (SB), writing, night walk</td>
</tr>
<tr>
<td>18-Feb</td>
<td>At La Selva</td>
<td>Analysis, writing</td>
<td>Analysis, writing</td>
<td>SIFP-5 symposium</td>
</tr>
<tr>
<td>19-Feb</td>
<td>At La Selva</td>
<td>Final ms due</td>
<td>Travel</td>
<td>Lec: Cons Bio (MA,JB)</td>
</tr>
<tr>
<td>20-Feb</td>
<td>To San Jose</td>
<td>Exploration</td>
<td>Travel</td>
<td>Free</td>
</tr>
<tr>
<td>21-Feb</td>
<td>To Cayman</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Morning</td>
<td>Afternoon</td>
<td>Evening</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------------</td>
<td>-----------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>21-Feb</td>
<td>Arrive from CR</td>
<td>Orientation: field station logistics and safety. Discussion about BIO FSP program to date and everyone’s expectations for LC segment.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22-Feb</td>
<td>Discussion about BIO FSP program to date and everyone’s expectations for LC segment. Free time to snorkel. Get BCD and regulator from Lowell.</td>
<td>Natural history discussion after dive. Coral Biology lecture Ellen.</td>
<td>Introduction to coral reefs: local geology and reef morphology (BT) Assign expert taxonomic groups. Natural history discussion before dinner.</td>
<td></td>
</tr>
<tr>
<td>23-Feb</td>
<td>SCUBA –shore dive at Cumber’s Cave (check dive)</td>
<td></td>
<td>Algae lecture (BT) Emilia, Elise.</td>
<td></td>
</tr>
<tr>
<td>24-Feb</td>
<td>Project 1 exploration 1-2 algae lab 4 pm Project 1 Discussion 5 pm Kali. Collect zooplankton.</td>
<td>Invertebrates lecture (RC) Colleen, Sammi Project 1 – solidified &amp; proposal.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-Feb</td>
<td>Project 1 starts SCUBA</td>
<td>Natural history discussion before dinner. Project 1 – begins. 5 pm Emma Camp.</td>
<td>Jimena, Amelia Herbivory (ZG) Fish Behavior (BT)</td>
<td></td>
</tr>
<tr>
<td>26-Feb</td>
<td>Project 1 Project 1</td>
<td>Fish ecology lecture (BT) Isa, Vicki. Sponge lecture (ZG).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27-Feb</td>
<td>SCUBA</td>
<td>Project 1</td>
<td>Eliza, Molly. Food webs (RC).</td>
<td></td>
</tr>
<tr>
<td>28-Feb</td>
<td>Project 1 Project 1</td>
<td>Ball hockey Tyler, Jill. Peer review discussion (See Bb).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Mar</td>
<td>SCUBA pending progress on papers</td>
<td>Project 1 5 pm Seth.</td>
<td>Project 1 – Symposium Project 1 - DUE.</td>
<td></td>
</tr>
<tr>
<td>2-Mar</td>
<td>Project 2 exploration</td>
<td>4 pm Project 2 - Discussion.</td>
<td>Authorship discussion (See Bb).</td>
<td></td>
</tr>
<tr>
<td>3-Mar</td>
<td>Project 2 starts Project 2</td>
<td>Project 2</td>
<td>R workshop.</td>
<td></td>
</tr>
<tr>
<td>4-Mar</td>
<td>SCUBA pending progress on papers</td>
<td>Project 2</td>
<td>Graduate school discussion &amp; Women in science.</td>
<td></td>
</tr>
<tr>
<td>5-Mar</td>
<td>Project 2</td>
<td>Project 2</td>
<td>Marine protected areas lecture.</td>
<td></td>
</tr>
<tr>
<td>6-Mar</td>
<td>SCUBA</td>
<td>Project 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Mar</td>
<td>Project 2</td>
<td>Project 2</td>
<td>Ball hockey or SCUBA night dive – Cumber’s Caves.</td>
<td></td>
</tr>
<tr>
<td>8-Mar</td>
<td>Project 2</td>
<td>Project 2</td>
<td>Project 2 - Symposium Project 2 - DUE.</td>
<td></td>
</tr>
<tr>
<td>9-Mar</td>
<td>Final edits</td>
<td>Final edits</td>
<td>SCUBA night dive – Cumber’s Caves.</td>
<td></td>
</tr>
<tr>
<td>10-Mar</td>
<td>Pack &amp; clean up Pack &amp; clean up Email all FINAL materials, including metadata to both TAs.</td>
<td>Pack &amp; clean up</td>
<td>Barbeque.</td>
<td></td>
</tr>
<tr>
<td>11-Mar</td>
<td>Depart for Grand</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Papers for Student Presentations: Costa Rica

<table>
<thead>
<tr>
<th>Site</th>
<th>Lecture</th>
<th>Student</th>
<th>Paper to Present</th>
</tr>
</thead>
<tbody>
<tr>
<td>LaSel</td>
<td>Wildcard</td>
<td>Ellen Irwin</td>
<td>Novotny, V. et al. 2006. Why are there so many species of herbivorous insects in tropical rainforests? <em>Science</em> 313: 115. (*See also: &quot;Crafting the pieces of the diversity jigsaw puzzle&quot; by R.L. Kitching from the same issue).</td>
</tr>
</tbody>
</table>
# Papers for Student Presentations: Little Cayman

<table>
<thead>
<tr>
<th>Student</th>
<th>Lecture</th>
<th>Paper</th>
</tr>
</thead>
</table>
# Species Lists

## Palo Verde

<table>
<thead>
<tr>
<th>Plants</th>
<th>Arthropods</th>
<th>Birds Continued</th>
<th>Birds Continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACANTHACEAE</td>
<td>Crematogaster crinosa</td>
<td>Black-headed Trogan</td>
<td>Roadside Hawk</td>
</tr>
<tr>
<td>Avicennia germinans</td>
<td>Myrmeleon crudelis</td>
<td>Blue winged teal</td>
<td>Rock Pigeon</td>
</tr>
<tr>
<td>ARECACEAE</td>
<td>Pepsis sp.</td>
<td>Boat-billed Flycatcher</td>
<td>Roseate spoonbill</td>
</tr>
<tr>
<td>Bactris sp.</td>
<td>Pseudomyrmex ferrugineus</td>
<td>Brown Pelican</td>
<td>Rufus-collared Sparrow</td>
</tr>
<tr>
<td>BIGNONIACEAE</td>
<td>Pseudomyrmex flavicornis</td>
<td>Cattle Egret</td>
<td>Scarlet Macaw</td>
</tr>
<tr>
<td>Crescentia alata</td>
<td>Scorpion</td>
<td>Cinnamon hummingbird</td>
<td>Snowy egret</td>
</tr>
<tr>
<td>BOMBACACEAE</td>
<td>Siproeta stelenes</td>
<td>Common Pauraque</td>
<td>Spectacled Owl</td>
</tr>
<tr>
<td>Bombacopsis quinatum</td>
<td>Trigona sp.</td>
<td>Crane hawk</td>
<td></td>
</tr>
<tr>
<td>BURSERACEAE</td>
<td>Costa Rican Tiger Rump tarantula</td>
<td>Double striped thick-knee</td>
<td></td>
</tr>
<tr>
<td>Bursera simaruba</td>
<td>Cave Roach</td>
<td>Ferruginous Pygmy-Owl</td>
<td></td>
</tr>
<tr>
<td>COMBRETACEAE</td>
<td>Solfugid?</td>
<td>Fulvous whistling duck</td>
<td></td>
</tr>
<tr>
<td>Laguncularia racemosa</td>
<td>Amblypygid</td>
<td>Glossy ibis</td>
<td></td>
</tr>
<tr>
<td>Terminalia catappa</td>
<td></td>
<td>Gray Hawk</td>
<td></td>
</tr>
<tr>
<td>FABACEAE</td>
<td></td>
<td>Great blue heron</td>
<td></td>
</tr>
<tr>
<td>Acacia collinsi</td>
<td></td>
<td>Great Curassow</td>
<td></td>
</tr>
<tr>
<td>Acacia cornigera</td>
<td></td>
<td>Great Egret</td>
<td></td>
</tr>
<tr>
<td>Bauhinia sp.</td>
<td></td>
<td>Great Kiskadee</td>
<td></td>
</tr>
<tr>
<td>Mimosa pudica?</td>
<td></td>
<td>Great-tailed Grackle</td>
<td></td>
</tr>
<tr>
<td>Parkinsonia aculeata</td>
<td>Rabbit (cotton tail?)</td>
<td>Green heron</td>
<td></td>
</tr>
<tr>
<td>MARANTACEAE</td>
<td>Coatiundi</td>
<td>Groove-billed Ani</td>
<td></td>
</tr>
<tr>
<td>Thalia sp.</td>
<td>Lesser White-lined bat</td>
<td>Hoffman’s Woodpecker</td>
<td></td>
</tr>
<tr>
<td>PELLICIERACEAE</td>
<td>Peccary</td>
<td>Inca Dove</td>
<td></td>
</tr>
<tr>
<td>Pelliciera rhizophorae</td>
<td>Procyon lotor (raccoon)</td>
<td>Jabiru</td>
<td></td>
</tr>
<tr>
<td>PONTEDERIACEAE</td>
<td>White tailed deer</td>
<td>Least Grebe</td>
<td></td>
</tr>
<tr>
<td>Eichhornia crassipes</td>
<td>Capuchin</td>
<td>Limpkin</td>
<td></td>
</tr>
<tr>
<td>TYPHACEAE</td>
<td>Howler</td>
<td>Little blue heron</td>
<td></td>
</tr>
<tr>
<td>Typha latifolia</td>
<td>Spider monkey</td>
<td>Mangrove cuckoo</td>
<td></td>
</tr>
<tr>
<td>Mammals</td>
<td>Agouti</td>
<td>Mangrove swallow</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Montezuma Oropendula</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Muscovy Duck</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nighthawk (spp?)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Northern jacana</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Northern Shoveler</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Osprey</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pale-billed Woodpecker</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peregrine Falcon</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Purple gallinule</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mollusks</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pomacea flagellata flagellata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reptiles/Amphibians</td>
<td>Birds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ameiva festiva</td>
<td>American coot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boa constrictor</td>
<td>Anhinga</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crocodylus americanus</td>
<td>Baltimore oriole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ctenosaura similis</td>
<td>Barn swallow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eleutherodactylus</td>
<td>Black bellied whistling duck</td>
<td></td>
<td></td>
</tr>
<tr>
<td>caryophyllaceus</td>
<td>Black necked stilt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iguana iguana</td>
<td>Black Vulture</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norops cupreus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giant Toad</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Species Lists

### Santa Rosa

**Plants**
- COMBRETACEAE
- Conocarpus erectus

**Arthropods**
- Phasmatodea

**Reptiles/Amphibians**
- Norops cupreus
- Ctenosaura similis
- Crocodylus americanus
- Lepidochelys olivacea

**Birds**
- Anhinga
- Brown Booby
- Brown Pelican
- Crested Caracara
- Great Black-Hawk
- Great Curassow
- Green-breasted Mango
- Groove-billed Ani
- Least Sandpiper
- Magnificent Frigatebird
- Mangrove Black-Hawk
- Orange-fronted Parakeet
- Prothonotary Warbler
- Roadside Hawk
- Sanderling
- White Ibis
- Green Kingfisher
- Collared Kingfisher

### Monteverde

**Plants**
- ARACEAE
- ARALIACEA
- BEGONIACEAE
- CECROPIACEAE
- CLUSIACEAE
- HELICONIACEAE
- LAURACEAE
- PIPERACEAE
- RUBIACEAE
- ZINGIVERACEAE

**Arthropods**
- Agelaia panamensis
- Millipede spp.
- Scolytidae
- Tachinidae

**Reptiles/Amphibians**
- Bothriechis lateralis
- Rana sp.
- Scelophorus

**Birds**
- Black Vulture
- Black-crowned Motmot
- Brown Jay
- Central American Pygmy-owl
- Collared Redstart
- Common Bush-tanager
- Great-tailed Grackle
- Purple-throated Mountain-gem
- Slate-throated Redstart
- Turkey Vulture

### Cuerici

**Plants**
- ALSTROMERIACEAE
- Bomarea sp.
- ARACEAE
- Anthurium sp.
- Monstera sp.
- Philodendron sp.
- ARALIACEAE
- Oreopanax pycnocarpus
- Shefflera
- ARECACEAE
- Chamaedorea
- Geonoma
- Prestoea accuminata
- ASTERACEAE
- Senecio grandifolius
- BALANOPHORACEAE
- BEGONIACEAE
- Begonia involucrata
- BETULACEAE
- Alnus acuminata
- CAMPANULACEAE
- Centropogon sp.
- CORNACEAE
- Cornus sp.
- CUCURBITACEAE
- CUNONIACEAE
- Weinmannia pinnata
- ERICACEAE
- Cavendishia bracteata
- LAURACEAE
- LYCOPODIACEAE
- Lycopodium sp.
- MELASTOMATAECEAE
- MYRTACEAE
- PAPAVERACEAE
- Bocconia frutescens

**Arthropods**
- Dione moneta
- Eciton burchelli
- Eciton hamatum
- Nasutitermes ephrata

**Birds**
- Acorn Woodpecker
- Black Vulture
- Black-capped Flycatcher
- Black-cheeked Warbler
- Black-faced Solitaire
- Collared Redstart
- Common Bush-tanager
- Dusky Nightjar
- Large-footed Finch
- Laughing Falcon
- Mountain Robin
- Ruddy-capped Nightingale-thrush
- Rufous-collared Sparrow
- Sooty Robin
- Sooty-capped Bush-Tanager
- Spot-crowned Woodcreeper
- Turkey Vulture
- Volcano Hummingbird
- Wilson's Warbler

**Mammals**
- Raccoon
- Puma
- Tapir
- Agouti
- White-tailed Deer
- Coati
- Whale

**Plants continued**
- POACEAE
- Chusquea longifolia
- Chusquea subtessellata
- Chusquea patens
- WINTERACEAE
- Drymis granadensis

**Reptiles/Amphibians**
- Dione moneta
- Eciton burchelli
- Eciton hamatum
- Nasutitermes ephrata

**Birds**
- Acorn Woodpecker
- Black Vulture
- Black-capped Flycatcher
- Black-cheeked Warbler
- Black-faced Solitaire
- Collared Redstart
- Common Bush-tanager
- Dusky Nightjar
- Large-footed Finch
- Laughing Falcon
- Mountain Robin
- Ruddy-capped Nightingale-thrush
- Rufous-collared Sparrow
- Sooty Robin
- Sooty-capped Bush-Tanager
- Spot-crowned Woodcreeper
- Turkey Vulture
- Volcano Hummingbird
- Wilson's Warbler

**Mammals**
- Raccoon
- Puma
- Tapir
- Agouti
- White-tailed Deer
- Coati
- Whale
Species Lists

**Plants**
MALVACEAE
Apeiba sp.
PASSIFLORACEAE
Passiflora sp.
RUBIACEAE
Psychotria sp.

**Arthropods**
BRUCHIDAE
Cicadas
Heliconius parchinus
Heliconius sara
Long horn beetle
Nephila clavipes

**Reptiles/Amphibians**
Basilisk
Brilliant forest frog
Crocodile
Norops spp.
Whiptail Lizard

**Mammals**
Three-toed Sloth
Baird's Tapir
Collared Peccary
Howler Monkey
Squirrel Monkey
Spider Monkey
White-faced Capuchin
Tamandua

**Fish**
Bull Shark
Cat-eye Fish

**Birds**
Baird's Trogan
Bare-throated Tiger-Heron
Black-faced Antthrush
Black-hooded Antshrike
Black-throated Trogan

**Corcovado**
Malvaceae
Blue-crowned Manakin
Blue-gray Tanager
Blue-throated Goldentail
Bronzy Hermit
Brown Pelican
Cattle Egret
Cherrie's Tanager
Chestnut-backed Antbird
Chestnut-mandibled Toucan
Crane Hawk
Crested Caracara
Crested Guan
Golden-crowned Spadebill
Gray-headed Tanager
Great Curassow
Great Egret
Great Kiskadee
Great Tinamou
Great-tailed Grackle
Green Heron
Green Kingfisher
Least Sandpiper
Little Blue Heron
Little Tinamou
Long-billed Hermit
Magnificent Frigatebird
Mangrove Black-Hawk
Mealy Parrot
Northern Waterthrush
Osprey
Pale-billed Woodpecker
Plain Xenops
Red-crowned Woodpecker
Red-lored Parrot
Ringed Kingfisher
Roadside Hawk
Rock Pigeon

**Birds continued**
Ruddy Turnstone
Ruddy Woodcreeper
Scarlet Macaw
Semipalmated Plover
Semipalmated Sandpiper
Short-billed Pigeon
Slaty-tailed Trogan
Snowy Egret
Southern Rough-winged Swallow
Spotted Sandpiper
Stripe-throated Hermit
Summer Tanager
Swallow-tailed Kite
Tawny-winged Woodcreeper
Three-wattled Bellbird
Tricolored Heron
Violaceous Trogan
Violet-crowned Woodnymph
Whimbrel
White Ibis
White-collared Swift
White-shouldered Tanager
White-throated Shrike-Tanager
White-whiskered Puffbird
Yellow-crowned Caracara
### Species Lists

#### Las Cruces

<table>
<thead>
<tr>
<th>Plants</th>
<th>Plants continued</th>
<th>Plants</th>
<th>La Selva</th>
<th>Birds continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRYOPHYTA</td>
<td>ASPARAGACEAE</td>
<td>FABACEAE</td>
<td></td>
<td>White-breasted Wood-wren</td>
</tr>
<tr>
<td>LYCOPHYTA</td>
<td>Agave sp.</td>
<td>Inga alba</td>
<td></td>
<td>Green-hooded Creeper</td>
</tr>
<tr>
<td>Lycopodium</td>
<td>ARAEACEAE</td>
<td>Eciton burchelli</td>
<td></td>
<td>Black-cheeked Woodpecker</td>
</tr>
<tr>
<td>PTERIDOPHYTA</td>
<td>Monstera sp.</td>
<td>Fulgora lateraria</td>
<td></td>
<td>Masked Tityra</td>
</tr>
<tr>
<td>CYATHEACEAE</td>
<td>ARECACEAE</td>
<td>Paraponera clavata</td>
<td></td>
<td>Plain Brown Woodcreeper</td>
</tr>
<tr>
<td>CYCADOPHYTA</td>
<td>BROMELIACEAE</td>
<td></td>
<td></td>
<td>Bay Wren</td>
</tr>
<tr>
<td>CONIFEROPHYTA</td>
<td>CYCLANTHACEAE</td>
<td></td>
<td></td>
<td>Northern Barred Woodcreeper</td>
</tr>
<tr>
<td>ANTHOPHYTA</td>
<td>HELICONIACEAE</td>
<td></td>
<td></td>
<td>White-billed Woodcreeper</td>
</tr>
<tr>
<td>DICOTS</td>
<td>LILINACEAE</td>
<td></td>
<td></td>
<td>Swainson's Hawk</td>
</tr>
<tr>
<td>ANACARDIACEAE</td>
<td>MARANTACEAE</td>
<td></td>
<td></td>
<td>Semi-plumbeous Hawk</td>
</tr>
<tr>
<td>Anacardium sp.</td>
<td>MUSACEAE</td>
<td></td>
<td></td>
<td>White-fronted Parrot</td>
</tr>
<tr>
<td>ASTERACEAE</td>
<td>Musa sp.</td>
<td></td>
<td></td>
<td>Golden-hooded Tanager</td>
</tr>
<tr>
<td>BEGONIACEAE</td>
<td>ORCHIDACEAE</td>
<td></td>
<td></td>
<td>Spot-crowned Euphonia</td>
</tr>
<tr>
<td>BETULACEAE</td>
<td>POACEAE</td>
<td></td>
<td></td>
<td>Blue-gray Grassquit</td>
</tr>
<tr>
<td>Alnus sp.</td>
<td>Chusquea sp.</td>
<td></td>
<td></td>
<td>Clay-colored Robin</td>
</tr>
<tr>
<td>BURSERACEAE</td>
<td>STRELITZIACEAE</td>
<td></td>
<td></td>
<td>Song Wren</td>
</tr>
<tr>
<td>Bursera sp.</td>
<td>ZINGIBERACEAE</td>
<td></td>
<td></td>
<td>Squirrel Cuckoo</td>
</tr>
<tr>
<td>CACTACEAE</td>
<td></td>
<td></td>
<td></td>
<td>White-collared Mannakin</td>
</tr>
<tr>
<td>CLUSIACEAE</td>
<td></td>
<td></td>
<td></td>
<td>Yellow-bellied Elaenia</td>
</tr>
<tr>
<td>ERICACEAE</td>
<td></td>
<td></td>
<td></td>
<td>Band-backed Wren</td>
</tr>
<tr>
<td>EUPHORBIACEAE</td>
<td></td>
<td></td>
<td></td>
<td>Great Green Macaw</td>
</tr>
<tr>
<td>FAGACEAE</td>
<td></td>
<td></td>
<td></td>
<td>Brown-hooded Parrot</td>
</tr>
<tr>
<td>Quercus sp.</td>
<td></td>
<td></td>
<td></td>
<td>Slate-headed Tody-flycatcher</td>
</tr>
<tr>
<td>LAURACEAE</td>
<td></td>
<td></td>
<td></td>
<td>Black Vulture</td>
</tr>
<tr>
<td>MELASTOMATACEAE</td>
<td></td>
<td></td>
<td></td>
<td>Anhinga</td>
</tr>
<tr>
<td>MORACEAE</td>
<td></td>
<td></td>
<td></td>
<td>Bare-throated Tiger-heron</td>
</tr>
<tr>
<td>Ficus sp.</td>
<td></td>
<td></td>
<td></td>
<td>Blue-gray Tanager</td>
</tr>
<tr>
<td>PASSIFLORACEAE</td>
<td></td>
<td>Chestnut-mandibled Toucan</td>
<td></td>
<td>Violaceous Trogan</td>
</tr>
<tr>
<td>Passiflora sp.</td>
<td></td>
<td>Cherrie's Tanager</td>
<td></td>
<td>Bright-rumped Attila</td>
</tr>
<tr>
<td>PIPERACEAE</td>
<td></td>
<td>Keel-billed Toucan</td>
<td></td>
<td>Purple-crowned Fairy</td>
</tr>
<tr>
<td>Piper sp.</td>
<td></td>
<td>Rufous-tailed Hummingbird</td>
<td></td>
<td>Broad-billed Motmot</td>
</tr>
<tr>
<td>RUBIACEAE</td>
<td></td>
<td>Montezuma Oropendola</td>
<td></td>
<td>Turkey Vulture</td>
</tr>
<tr>
<td>Coffea sp.</td>
<td></td>
<td></td>
<td></td>
<td>Snowy Egret</td>
</tr>
<tr>
<td>SOLANACEAE</td>
<td></td>
<td></td>
<td></td>
<td>Long-tailed Tyrant</td>
</tr>
<tr>
<td>MONOCOTS</td>
<td></td>
<td></td>
<td></td>
<td>Great Potoo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chestnut-headed Oropendola</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Black-faced Grosbeak</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Boat-billed Flycatcher</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rufous Motmot</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cattle Egret</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chestnut-colored Woodcreeper</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pale-billed Woodpecker</td>
</tr>
</tbody>
</table>

#### Reptiles/Amphibians

- Strawberry Frog
- Black River Turtle
- Eyelash Pit Viper
- Hog-nosed Pit Viper
- Fer-de-lance
- Cane Toad
- Bird-eating Snake
- Whiptail Lizard
- Anole (Norops spp.)
- Basilisk

#### Fish

- Anhinga
- Machaca
- Violaceous Trogan
- Bright-rumped Attila
- Purple-crowned Fairy
- Broad-billed Motmot
- Turkey Vulture
- Snowy Egret

#### Mammals

- Two-toed Sloth
- Collared Peccary
- Ocelot
- Spider Monkey
- Howler Monkey
- White-faced Capuchin

#### Birds

- Chestnut-mandibled Toucan
- Cherrie's Tanager
- Keel-billed Toucan
- Rufous-tailed Hummingbird
- Montezuma Oropendola
- Long-tailed Tyrant
- Black-faced Grosbeak
- Boat-billed Flycatcher
- Tropical Kingbird
- Great Curassow
- Collared Aracari
### Species Lists

**La Selva continued**

**Birds continued**
- White-necked Jacobin
- Red-capped Mannakin
- Rufous Mourner
- Black-crowned Oriole
- Snowy Cotinga
- Mealy Parrot
- Slaty-breasted Tinamou
- Crested Guan
- Short-billed Pigeon
- Ruddy Ground-dove
- Common Pauraque
- White-necked Puffbird
- Buff-throated Saltator
- Swainson’s Thrush
- Social Flycatcher
- Great Kiskadee
- Western Slaty-Antshrike
- Slaty-tailed Trogon
- Long-billed Hermit
- Gray-cheeked Dove

**Plants**
- grape tree
- mangrove

**Arthropods**
- Blattodea
- Ants
- No-see-ums

**Reptiles/Amphibians**
- iguana
- Curly-tailed Lizard (*Leiocephalus carinatus granti*)
- Norops spp.
- Hawksbill turtle (*Eretmochelys imbricata*)

**Birds**
- Yellow-crowned Night Heron
- West Indian Whistling-Duck
- Magnificent Frigatebird
- Brown Pelican
- Royal Tern
- Bananaguit
- Yellow Warbler
- Smooth-billed Ani
- Cattle Egret
- Osprey
- Northern Mockingbird
- Merlin
- Red-footed Booby
- American Kestrel

**Mammals**
- Homo sapiens sapiens
- feral cat

**Fish**
- Barbfish (*Scorpaena brasiliensis*)
- Green razorfish (juvenile) (*Xyrichthys splendens*)
- Spotted scorpionfish (*Scorpaena plumieri*)
- Foureye butterflyfish (*Chaetodon capistratus*)
- Stoplight parrotfish (*Sparisoma viride*)
- Nassau grouper (*Epinephelus striatus*)
- Dusky damselfish (*Stegastes adustus*)
- Beaugregory damselfish (*Stegastes leucosticus*)

**Fish continued**
- Bicolor damselfish (*Stegastes partitus*)
- Yellowtail damselfish (*Microspathodon chrysurus*)
- Cocoa damselfish (*Stegastes variabilis*)
- Rainbow parrotfish (*Sparus guacamaia*)
- Queen parrotfish (*Sparus vetula*)
- Spotted moray (*Gymnothorax moringa*)
- Brown garden eel (*Heteroconger longissimus*)
- Southern stingray (*Dasyatis sabina*)
- Yellow goatfish (*Malacanthus plumieri*)
- Sand tilefish (*Malacanthus plumieri*)
- Goldspot goby (*Gnatholepis mutilatus*)
- Black durgon (*Melichthys niger*)
- Squirrelfish (*Holocentrus adscensionis*)
- Blue chromis (*Chromis cyanea*)
- Whitespotted filefish (*Cantherhines macrocerus*)
- Scrawled filefish (*Aluterus scriptus*)
- Slippery dick (*Halichoeres bivittatus*)
- Queen triggerfish (*Balistes vetula*)
- Bluehead wrasse (*Thalassoma bifasciatum*)
- Sergeant major (*Abudelfuf saxatilis*)
- Schoolmaster (*Lutjanus apodus*)
- Yellowtail snapper (*Ocyurus chrysurus*)
- French grunt (*Haemulon flavolineatum*)
- Sharpnose puffer (*Canthigaster rostrata*)
- French angelfish (*Pomacanthus paru*)
- Gray angelfish (*Pomacanthus arcuatus*)
- Porcupinefish (*Diodon hystrix*)
- Whitefin sharksucker (*Echeneis neucratoides*)
- Great barracuda (*Sphyraena barracuda*)
- Tarpon (*Megalops atlanticus*)
- Queen angelfish (*Holacanthus ciliaris*)
- Rock beauty (*Holacanthus tricolor*)
- Banded butterflyfish (*Chaetodon striatus*)
- Longfin damselfish (*Stegastes diencaeus*)
- Goldline blenny (*Malacanthus aurolineatus*)
- Redlip blenny (*Ophioblennius macclurei*)
- Spotted drum (*Echeneis neucratoides*)
- Peacock flounder (*Bothus lunatus*)
## Species Lists

### Little Cayman continued

<table>
<thead>
<tr>
<th>Fish continued</th>
<th>Fish continued</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purplemouth moray Gymnothorax vicinus</td>
<td>Dusky squirrelfish Sargocentron vexillarium</td>
</tr>
<tr>
<td>Spotfin butterflyfish Chaetodon ocellatus</td>
<td>Neon goby Elacatinus oceanops</td>
</tr>
<tr>
<td>Longnose butterflyfish Prognathodes aculeatus</td>
<td>Yellowline goby Elacatinus horsti</td>
</tr>
<tr>
<td>Blue tang Acanthurus coeruleus</td>
<td>Cleaning goby Elacatinus genie</td>
</tr>
<tr>
<td>Ocean surgeonfish Acanthurus tructus</td>
<td>Spotted goby Coryphopterus puncticipelorpus</td>
</tr>
<tr>
<td>Doctorfish Acanthurus chirurgus</td>
<td>Yellowhead jawfish Opistognathus aurifrons</td>
</tr>
<tr>
<td>Bar jack Caranx ruber</td>
<td>Banded jawfish Opistognathus macronathus</td>
</tr>
<tr>
<td>Horse-eye jack Caranx latus</td>
<td>Maculated flounder Bothus maculiferus</td>
</tr>
<tr>
<td>Keeltail needlefish Platybelone argalus</td>
<td>Atlantic trumpetfish Aulostomus maculatus</td>
</tr>
<tr>
<td>Houndfish Tylosurus crocodilus</td>
<td>Bridled burrfish Chilomycterus antennatus</td>
</tr>
<tr>
<td>Atlantic flyingfish Cheilopogon melanurus</td>
<td>Spotted burrfish Chilomycterus aringa</td>
</tr>
<tr>
<td>Guaguanche Sphyraena guanchancho</td>
<td>Scrawled cowfish Acanthostracion quadricornis</td>
</tr>
<tr>
<td>Bonefish Albula vulpes</td>
<td>Smooth trunkfish Lactophrys triqueter</td>
</tr>
<tr>
<td>Silver porgy Diplodus argenteus</td>
<td>Spotted trunkfish Lactophrys bicaudalis</td>
</tr>
<tr>
<td>Saucereye porgy Calamus calamus</td>
<td>Spotted goatfish Pseudopeneus maculatus</td>
</tr>
<tr>
<td>Bluespotted grunt Haemulon sciurus</td>
<td>Nurse shark Ginglymostoma cirratum</td>
</tr>
<tr>
<td>Caesar grunt Haemulon carbonarium</td>
<td>Reef shark Carcharhinus perezii</td>
</tr>
<tr>
<td>Margate (White) Haemulon album</td>
<td>Yellow stingray Urobatis jamaicensis</td>
</tr>
<tr>
<td>Mutton snapper Lutjanus analis</td>
<td>Spotted eagle ray Aetobatus narinari</td>
</tr>
<tr>
<td>Threespot damselfish Stegastes planifrons</td>
<td>Chain moray</td>
</tr>
<tr>
<td>Brown chromis Chromis multilineata</td>
<td>Marine Invertebrates</td>
</tr>
<tr>
<td>Graysby Cephalopholis cruentada</td>
<td>Caribbean spiny lobster Panulirus argus</td>
</tr>
<tr>
<td>Red hind Epinephelus guttatus</td>
<td>Reef urchin Echinometra viridis</td>
</tr>
<tr>
<td>Coney Cephalopholis fulva</td>
<td>Long-spined sea urchin Diadema antillarum</td>
</tr>
<tr>
<td>Tiger grouper Mycteroperca tigris</td>
<td>Leopard flatworm Pseudoceros pardinis</td>
</tr>
<tr>
<td>Yellowfin grouper Mycteroperca venenosa</td>
<td>Banded coral shrimp Stenopelmatus hispidus</td>
</tr>
<tr>
<td>Greater Soapfish Rypticus saponaceus</td>
<td>Queen conch (Strombus gigas)</td>
</tr>
<tr>
<td>Fairy basslet Gramma loreto</td>
<td>hermit crab (sp?)</td>
</tr>
<tr>
<td>Blue parrotfish Scarus coeruleus</td>
<td>Christmas tree worm Spirobranchus giganteus</td>
</tr>
<tr>
<td>Midnight parrotfish Scarus coelestinus</td>
<td>Brown fanworm Notaulax nudicollis</td>
</tr>
<tr>
<td>Princess parrotfish Scarus taeniopeterus</td>
<td>Split-crown feather duster Anamobaea orstedii</td>
</tr>
<tr>
<td>Striped parrotfish Scarus iseri</td>
<td>Christmas tree hydroid Halocordyle disticha</td>
</tr>
<tr>
<td>Yellowtail parrotfish Sparisoma rubripinne</td>
<td>Giant anemone Condylactis gigantea</td>
</tr>
<tr>
<td>Bluelip parrotfish Cryptotomus roseus</td>
<td>Corkscrew anemone Bartholomea annulata</td>
</tr>
<tr>
<td>Spanish hogfish Bodianus rufus</td>
<td>Knobby anemone Ragactis lucida</td>
</tr>
<tr>
<td>Creole wrasse Clepticus parrae</td>
<td>Turtle grass anemone Viatrix globulifera</td>
</tr>
<tr>
<td>Yellowhead wrasse Halichoeres garnoti</td>
<td>Red warty anemone Bunodosoma granulifera</td>
</tr>
<tr>
<td>Longspine squirrelfish Holocentrus rufus</td>
<td></td>
</tr>
</tbody>
</table>

---

xiii
Species Lists

**little cayman continued**

**Marine Invertebrates continued**

- Sponge anemone
- White encrusting zoanthid Palythoa caribaeorum
- Banded tube-dwelling anemone Arachnanthis nocturnus
- Ctenophores?
- Bicolored flatworm Pseudoceros bicolor
- Bearded fireworm Hermodice carunculata
- Southern lugworm Arenicola cristata
- Variegated feather duster Bispira variegata
- Yellow fanworm Notaulax occidentalis
- Black-spotted feather duster Branchiomma nigromaculata
- Star horseshoe worm Pomatostegus stellatus
- Spaghetti worm Eupolympnia crassicornis
- Pederson cleaner shrimp Periclimenes pedersoni
- Sculptured slipper lobster Parribacus antarcticus
- Spotted spiny lobster Panulirus guttatus
- Plumed hairy crab Pilumnus floridanus
- Rough box crab Calappa gallus
- Blotched swimming crab Portunus spinimanus
- Blue crabs Callinectes spp.
- Nimble spray crab Percnon gibbesi
- Green clinging crab Mithrax sculptus
- Yellowline arrow crab Stenorhynchus seticornis
- Ciliated false squilla Pseudosquilla ciliate
- Isopods (Cymothoidae)
- Mysid shrimp Mysidium spp.
- Crown conch Melongena corona
- West Indian starsnail Lithopoma tectum
- Chocolate-lined topsnail Calliostoma javanicum
- Angulate wentletrap Epitonium angulatum
- Flamingo tongue Cyphoma gibbosum
- Spiny fileclam Lima lima
- Sunrise tellin Tellina radiata
- Caribbean reef squid Sepioteuthis sepioidea
- Longfin squid Loligo pealei
- Caribbean reef octopus Octopus birareus
- Golden crinoid Davidaster rubiginosa
- Beaded crinoid Davidaster discoidea
- Cushion sea star Oreaster reticulatus

**Marine Invertebrates continued**

- Sponge brittle star Ophiurothrix suensonii
- Blunt-spined brittle star Ophiocoma echinata
- Rock-boring urchin Echinometra lucunter
- Slate-pencil urchin Euclidaris tribuloides
- Donkey dung sea cucumber Holothuria mexicana
- Warty seacat Dolabrida dolabrifera

**Coral**

- Sea Fans (Gorgonia spp.)
- Symmetrical Brain Coral (Diploria strigosa)
- Lettuce Coral (Agaricia agaricites)
- Massive Starlet Coral (Siderastrea siderea)
- Black Sea Rod (Plexaura homomalla)
- Blade Fire Coral (Millepora complanata)
- Lesser Starlet Coral (Siderastrea radians)
- Elkhorn Coral (Acropora palmata)
- Smooth Flower Coral (Eusimilia fastigia)
- Finger Coral (Porites porites)

**Sponges**

- nettled barrel sponge
- brown tube sponge
- Branching Tube Sponge (Pseudoceratina crassa)
- Scattered Pore Rope Sponge (Aplysina fulva)
- brown variable sponge
- Branching Vase Sponge (Callyspongia vaginalis)
- Pitted Sponge (Verongula rigida)
- Giant Barrel Sponge (Xestospongia muta)
- Yellow Tube Sponge (Aplysina fistularis)

**Algae and Marine Plants**

- mermaid’s wine glass
- lettuce algae?
- white scroll algae
- Turtle grass
- Halimeda incrassata
- Galaxaura oblongata
Table of Contents

Participants of the 2012 FSP i
Note from Professor Ayres ii
Costa Rica 2012 Schedule iii
Little Cayman 2012 Schedule iv
Papers for Student Presentations in Costa Rica v
Papers for Student Presentations in Little Cayman vi
Maps vii
Species lists viii - xiv

Palo Verde

**Enemy at the gates: possible evidence for dear enemy phenomenon in** *Crematogaster crinosa*. Seth A. Brown, Colleen P. Cowdery, Jimena Diaz, Elisabeth R. Seyferth, And Victoria D. H. Stein 1

**The dear enemy effect in** *Crematogaster crinosa*. Amelia F. Antrim, Tyler E. Billipp, Emilia H. Hull, Kali M. Pruss, Molly R. Pugh 5

**Two alarms for one tree? Differential response of** *Pseudomyrmex spinicola* **to multiple stimuli.** Gillian A.O. Britton, Samantha C. Dowdell, Eliza W. Huntington, Ellen T. Irwin, and Maria Isabel Regina D. Francisco 9

**Survivorship and resistance in the predator-prey interactions between ants and antlions.** Amelia F. Antrim, Tyler E. Billipp, Maria Isabel Regina D. Francisco, Elisabeth R. Seyferth, and Victoria D. Stein 13

**Investigating the effects of aposematism on predator avoidance.** Seth A. Brown, Samantha C. Dowdell, Eliza W. Huntington, Ellen T. Irwin, and Kali M. Pruss 18

**A producer-consumer relationship: nectar advertising in Eicchornia Crassipes.** Gillian A. O. Britton, Colleen P. Cowdery, Jimena Diaz, Emilia H. Hull, Molly R. Pugh 21

**Predator and alarm call response in capuchin monkeys.** Amelia F. Antrim and Kali M. Pruss 25
Differential evasive response to predator calls in auditive moths.  
Tyler E. Billipp, Samantha C. Dowdell, Maria Isabel Regina D. Francisco, Elisabeth R. Seyferth

One robber, two victims: exploitation of Apis Mellifera and Eichhornia Crassipes plant-pollination mutualism through nectar robbing. Gilliam A. O. Britton, Colleen P. Cowdery, Jimena Diaz, Emilia H. Hull, Eliza W. Huntington, Ellen T. Irwin

Monteverde

Environmental and life history tradeoff effects on fertility in Thelypteris ferns. Seth A. Brown and Elisabeth R. Seyferth

The effect of hummingbird size on territoriality and foraging strategy. Colleen P. Cowdery, Emilia H. Hull, Ellen T. Irwin, Molly P. Pugh, Maria Isabel Francisco

Benefits of flushing red for a tropical tree (Alfaroa costaricensis). Amelia F. Antrim, Tyler E. Billipp, Gillian A. O. Britton, Eliza W. Huntington, and Kali M. Pruss

The effect of anthropogenic inputs on benthic stream invertebrates in a tropical montane stream. Jimena Diaz, Samantha C. Dowdell, Victoria D. H. Stein

Cuerici

Optimizing small-scale trout farming: effects of tagging, diet, and water quality on Oncorhynchus mykiss. Amelia F. Antrim, Seth A. Brown, Samantha C. Dowdell, Maria Isabel Regina D. Francisco, and Molly R. Pugh

Flies and flowers: Investigation of fly aggregations within N. speciosa flowers. Tyler E. Billipp, Colleen C. Cowdery, and Victoria D. Stein

Effect of fish density on metabolism of Oncorhynchus mykiss fry. Jimena Diaz, Ellen T. Irwin, and Elisabeth R. Seyferth

Abiotic and biotic factors affecting the growth of Palma morada (Prestoea Acuminata) in regeneration project in Cuerici, Costa Rica. Gillian A. O. Britton, Emilia H. Hull, Eliza W. Huntington, and Kali M. Pruss
Corcovado

David takes down goliath: interactions between Eciton burchelli, Eciton Hamatum and Nasutitermes ephratae. Seth A. Brown, Jimena Diaz, Eliza F. Huntington, and Kali M. Pruss

Commensalism and tidal foraging in estuary birds of Corcovado National Park. Amelia F. Antrim and Samantha C. Dowdell

Costly signals: Measuring the cost of dewlap display by Norops lizards. Gillian A. O. Britton, Maria Isabel Regina D. Francisco, and Elisabeth R. Seyferth

Bigger is better but more demanding: kleptoparasites, males, and metabolic needs of Nephila clavipes. Ellen T. Irwin, Molly R. Pugh, and Victoria D. Stein

Habitat selection in euglossine bees in Corcovado, Costa Rica. Tyler E. Billipp, Colleen P. Cowdery, and Emilia H. Hull

La Selva

Honest signalling for territory and mate interactions in strawberry poison dart frogs (Oophaga pumilio). Colleen P. Cowdery, Eliza W. Huntington, and Ellen T. Irwin

Island biogeography: are Heliconias islands? Tyler Billipp and Seth A. Brown

Mite Proctolaelaps kirmsei negatively affect Hamelia patens and its hummingbird pollinators? Amelia F. Antrim, Jimena Diaz, Samantha C. Dowdell, Maria Fransisco, Emilia H. Hull

Sharing is caring: Foraging benefits in mixed-species flocks of toucans and oropendolas. Gillian A. O. Britton, Kali M. Pruss, Molly R. Pugh, Elisabeth R. Seyferth, and Victoria D. Stein
Little Cayman

Friends with benefits: Does schooling behavior enhance foraging in blue tang, *Acanthurus coeruleus*, during interactions with territorial damselfish? Samantha C. Dowdell, Maria Isabel Regina D. Francisco, Emilia H. Hull, and Molly R. Pugh

A human-induced trophic cascade: effects of conch harvesting on marine plants. Amelia Antrim, Gillian Britton, Colleen Cowdery, Vicky Stein, Ellen Irwin, Eliza Huntington

Fish preferentially attack allelopathic algae over non-allelopathic algae on the corals *Acropora palmata* and *Diploria strigosa*. Tyler E. Billipp, Seth A. Brown, Jimena A. Diaz, Kali M. Pruss, and Elisabeth R. Seyferth

Seeking sanctuary: empty conch shells as refugia in habitats of varying structural complexity. Emilia H. Hull, Ellen R. Irwin, Kali M. Pruss

Let the wild rumbles begin: de-escalation of conflict through acoustic and visual signals in mantis shrimp (*Neogonodactylus oerstedii*). Victoria D. Stein, Colleen P. Cowdery, and Tyler E. Billipp

Importance of habitat fragment size, disturbance, and connectivity: An exploration of species diversity in tropical tidal pools. Elisabeth R. Seyferth, Samantha C. Dowdell, Maria Isabel Regina D. Francisco, Jimena Diaz, and Gillian A.O. Britton

Turtle grass growth response to herbivory. Amelia F. Antrim, Seth A. Brown, Eliza W. Huntington, and Molly R. Pugh
ENEMY AT THE GATES: POSSIBLE EVIDENCE FOR DEAR ENEMY PHENOMENON IN

CREMATOGASTER CRINOSA

SETH A. BROWN, COLLEEN P. COWDERY, JIMENA DIAZ, ELISABETH R. SEYFERTH, AND
VICTORIA D. H. STEIN

Abstract: The cost of intraspecific conflict is an important factor driving phenotypic and behavioral changes within populations. The “dear enemy” phenomenon is hypothesized to reduce costs of repeated conflict through mutually lowered aggression between neighboring colonies or individuals. Here, we test this phenomenon in the ant Crematogaster crinosa by asking if intraspecific aggression of C. crinosa towards ants introduced from other trees’ colonies changes with the distance between colonies. Focal ants were non-aggressive towards ants of neighboring colonies but became aggressive very quickly as distance increased. This result suggests that the dear enemy phenomenon exists in C. crinosa acacia ants. The relationship could also be explained by decreased relatedness in relatively distant colonies or by the polydomous colonization behavior of C. crinosa.

Key words: Acacia collinsii, Crematogaster crinosa, dear enemy phenomenon, intraspecific aggression

INTRODUCTION

The cost of intraspecific conflict is an important factor driving behavioral changes within populations. Many well-studied organisms such as mice and deer use species-specific signaling to reduce the impact of territorial contests between rivals (Clutton-Brock and Albon 1979, Jones and Nowell 1989). A related mechanism documented in a wide range of organisms is the “dear enemy phenomenon” (DEP), in which neighboring colonies or individuals are less aggressive towards each other when compared to non-neighbors (Fisher 1954). Researchers hypothesize that mutually lowered aggression between established neighbors decreases the cost of repeated conflict, allowing increased territorial and resource defense from intrusions by wandering strangers (Rosell and Bjørkøyli 2002, Dimarco et al. 2009).

In Crematogaster crinosa (Hymenoptera: Formicidae), a facultative mutualist ant of the Central and South American tree Acacia collinsii (Fabaceae: Mimosoideae) that forms large polydomous colonies (Longino 2003), defense from invaders is especially important given the shelter, protein, and energy the host tree provides (Longino 2003). These resources take the form of hollowed stipular spines, Beltian bodies, and extra-floral nectaries, respectively (Janzen 1966, Longino 2003). There is little research available on the inter-colony interactions of C. crinosa. The DEP, if in place, would suggest that C. crinosa budgets its efforts so as to avoid unnecessary conflicts between colonies, freeing resources for other tasks. Aggressive defense of the host acacia and its resources should increase greatly when invading ants are from non-neighboring colonies rather than from neighboring colonies. We predicted that C. crinosa ants would act less aggressively towards conspecific ants from adjacent trees than from distant trees, and that the levels of aggression would increase as the distance between the focal tree and foreign ant source increased. This would provide support for the DEP in C. crinosa acacia ants.

METHODS

Collection of ants
From 8 am to 11:30 am on January 11, 2013, in Palo Verde National Park in the Guanacaste region of Costa Rica, we haphazardly chose focal *A. collinsii* trees (from a large stand) that hosted *C. Crinosa* and had main trunk diameters between 3 and 6 cm at a height of 1.5 meters. We used a list of computer-generated random numbers (between 0 and 25) to determine a set of random distances and directions from which to choose secondary

Table 1. Aggression scale used to rank the behavior of focal tree ants towards introduced ants

<table>
<thead>
<tr>
<th>Rank</th>
<th>Times observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introduced ant was ignored by focal tree ants</td>
</tr>
<tr>
<td>2</td>
<td>Focal tree ant(s) approached the introduced ant</td>
</tr>
<tr>
<td>3</td>
<td>Biting from focal tree ant(s)</td>
</tr>
<tr>
<td>4</td>
<td>Focal tree ant(s) ejected the introduced ant from the tree</td>
</tr>
<tr>
<td>5</td>
<td>Focal tree ant(s) killed the introduced ant</td>
</tr>
</tbody>
</table>

acacias similarly hosting *C. crinosa*; if the distance and direction did not lead directly to a suitable acacia, the nearest acacia fitting study parameters was used. Random numbers were generated using random.org’s Integer Generator via atmospheric noise.

We collected between one and three “foreign” ants from the distant tree at the height of 1.5 m and marked them with DayGlo Color Corporation orange or green fluorescent powder, either by placing them in a bag containing the powder or by transporting them on a stick coated with powder. We then placed one ant onto the focal tree at a 1.5 m height, in the shade and within 1 cm of multiple focal tree ants. We observed the behavior of ants from the focal tree towards the foreign ant for two minutes, and recorded the time at which the most aggressive behavior was observed. We then ranked the behavior on an aggression scale (Table 1). The aggression score was assigned based on the most aggressive behavior observed within the two minute window. Of our 30 trials, five were randomly assigned controls for which we removed a focal tree ant, marked it, held it an equal amount of time as in the experimental trials, returned it to the focal tree, and scored the aggression of other focal ants over the two minute period. This treatment was designed to

![Graph](image)

**Figure 1.** Intraspecific aggression in *Crematogaster crinosa* increases linearly with log$_{10}$-transformed distance between colonies. ($y = 1.7056 \cdot \log_{10} \text{Distance} + 1.89$) Aggression score was measured for each encounter between an ant from a distant tree and ants from a chosen focal tree within a two-minute observation window in Palo Verde National Park, Costa Rica.
control for any aggression that was caused by removing foreign ants from their tree and also to ensure that the fluorescent powder did not obscure scent recognition in focal tree ants.

**Statistical Analysis**

We used regression analysis in JMP 10.0 software to test the relationship between aggression score and distance from the focal tree. We log_{10} transformed distance from focal tree to ensure that our data met all of the assumptions of the regression analysis.

**RESULTS**

Ant aggression towards foreign ants increased with distance from the focal tree ($r^2 = 0.46$, $P=0.0002$, Figure 1).

**DISCUSSION**

The positive linear relationship between log_{10} distance and aggression shows that neighboring colonies of *C. crinosa* are treated with less aggression than non-neighboring colonies, providing support for the dear enemy phenomenon (DEP). Our results suggest that *C. crinosa* ants are capable of recognizing traits specific to their neighbors and of using this recognition to avoid unnecessary and costly conflict with those conspecifics closest to them while remaining highly aggressive towards invading strangers. The majority of acacia ant foraging happens on the surface of the host plant, lowering the potential competitive threat of neighboring colonies that gain resources from their own host plant (Carroll and Janzen 1973). If the DEP applies, lowering the rate of unnecessary costly conflict between neighboring colonies would be advantageous without necessitating the surrender of any resources.

An alternate explanation for the relationship we observed could be the presence of a relation gradient whereby ants from neighboring colonies are less likely to be genetically related as distance between colonies increase. Colony relatedness is significantly higher when separated by smaller distances than by larger distances because of the limits of ant dispersal (Turke et al. 2010) and ants from neighboring colonies could be less aggressive because they share genes responsible for influencing chemical recognition cues.

Our support for the DEP is based on the assumption that ants in adjacent acacias are members of different colonies. However, this assumption may be false given that *C. crinosa* tends to have large colonies and exhibit polydomous colony occupation (Longino 2003). This means that nearby acacias could have housed ants of the same colony as the focal tree. The lack of aggression between the focal ants and those we had considered to be foreign would not support the DEP, but would instead illustrate non-aggressive intra-colony interactions as observed by Bos et al. (2011).

Finally, our results could be explained by a gradient of genetic relatedness. Future experiments could use genetic analysis to definitively reveal the boundaries of colonies and illuminate whether ants in nearby colonies share genetic material. This research would reveal whether our results stem from genetically related colonies or large polydomous colonies rather than from unrelated colonies exhibiting DEP. The results of our study and further genetic analysis could elucidate the factors surrounding aggressive behavior in *C. crinosa*.

**ACKNOWLEDGEMENTS**

We would like to thank the staff of Palo Verde Biological Research Station for their support, R. Chaves-Ulloa for inspiring the experimental design, and R. Calsbeek and Z. Gezon for their feedback and assistance.

**AUTHOR CONTRIBUTIONS**

All authors contributed equally to experimental design, execution of experiment, and writing of manuscript.
LITERATURE CITED


THE DEAR ENEMY EFFECT IN *CREMATOGASTER CRINOSA*

AMELIA F. ANTRIM, TYLER E. BILLIPP, EMILIA H. HULL, KALI M. PRUSS, MOLLY R. PUGH

Faculty Editor: Ryan Calsbeek

Abstract: The “dear enemy” effect asserts that territorial organisms should display less aggressive behavior towards neighboring organisms than towards distant ones. We investigated the dear enemy effect in the acacia ant, *Crematogaster crinosa*, which protects its home acacia tree against disturbances in return for food and shelter. We measured the behaviors of the resident ants towards ants introduced individually from the nearest neighboring tree (neighbors), a distant tree (strangers), and the resident ants’ tree (control). Results suggested that ants from the focal tree were more aggressive towards ants from distant trees than from neighboring trees. Furthermore, the focal ants’ aggression level increased over time towards distant ants and decreased towards control and neighboring ants. Our results support the hypothesis that acacia ants are able to identify whether introduced ants are familiar (either from the same colony or a neighboring one) or distant and react accordingly, thus demonstrating the dear enemy effect. The ants’ ability to discriminate among individuals shows the complexity and cooperation that characterize ant colonies.

Key words: Acacia ants, *Crematogaster crinosa*, dear enemy effect, intraspecific aggression

INTRODUCTION

Resource defense is crucial to the fitness of territorial organisms. Although territorial defense is often a high priority, foraging and reproductive needs limit time and energy that can be allocated to defense. Territorial organisms can avoid costly conflict with neighbors by establishing shared boundaries instead of repeatedly contesting them. This phenomenon, called the “dear enemy” effect, predicts that these organisms will display less aggressive behavior towards neighbors than strangers (Temeles 1994). Accordingly, organisms must be able to distinguish neighbors from strangers on the basis of potential threat.

The dear enemy effect has been observed in colonial ant species, including *Acromyrmex octospinosus* and *Leptothorax nylanderi* (Jutsum et al. 1979; Heinze et al. 1996), but remains largely unexamined in arboreal ants, including acacia ants. The acacia ant *Crematogaster crinosa* will defend its home acacia tree from disturbances, including herbivory and competition with other plants, in exchange for valuable food and shelter (Rehr et al. 1973). Spatial overlap of acacia ant colonies may facilitate the formation of shared territorial boundaries, which could reduce aggression between colonies. Thus, as acacia ants defend against potential usurpers (Elton 1932), the resident ants are likely to respond more aggressively towards ants that are strangers than neighbors (Temeles 1994).

We hypothesized that *C. crinosa* would adhere to the dear enemy effect by exhibiting more aggressive behavior towards acacia ants from a distant tree than towards those from a neighboring tree.

METHODS

We conducted our experiment from 8am to 12pm on the morning of January 12, 2013, in a large stand of acacia trees in the tropical dry forest of Palo Verde National Park, Costa Rica. We haphazardly chose a focal tree inhabited by *C. crinosa*, then identified its nearest neighbor as well as a distant tree (defined as a tree housing the same species of acacia ants and growing 10-15 m away from the focal tree). We collected three ants from each tree (focal, nearest neighbor, and distant) and randomly introduced individual ants to the focal tree at heights ranging between 1.0-1.5 m off the ground.

For each trial, we observed the introduced ant for three minutes and ranked the focal ants’ behavior each time they came into contact with the introduced ant (Table 1). We also recorded the time the introduced ant first came in contact with a focal ant. Occasionally, the introduced ant would move out of our range of observation (higher than 2 m up the tree or inside a domatia), at which point we would end the trial and attempt to remove the introduced ant. We began the next trial approximately 30 seconds after the end of the...
previous trial. We repeated this procedure at five focal trees, making sure the trees were adequately spaced (>5m) to ensure independence of our observations.

Table 1. Rank of focal ants’ behavior on a scale from zero to five. Behavior ranked from zero to two was categorized as “non-aggressive” and behavior ranked from three to five was categorized as “aggressive”.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Times observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No contact with introduced ant</td>
</tr>
<tr>
<td>1</td>
<td>Brief contact with the introduced ant (less than 5s)</td>
</tr>
<tr>
<td>2</td>
<td>Prolonged contact with the introduced ant (more than 5s)</td>
</tr>
<tr>
<td>3</td>
<td>Biting or grappling with the introduced ant</td>
</tr>
<tr>
<td>4</td>
<td>Swarming or immobilizing the introduced ant</td>
</tr>
<tr>
<td>5</td>
<td>Expelling the introduced ant from the focal tree</td>
</tr>
</tbody>
</table>

Statistical Analysis
All statistical tests were performed using JMP 10.0 software. We performed an ANCOVA comparing the most aggressive response to each ant for all treatments with time as a covariate, followed by a Tukey’s HSD to determine the significance between each pairwise comparison. Since aggressive behavior in some trials was limited by delayed initial contact with focal ants, we included time to first encounter as a covariate. We divided the trials into one-minute segments and conducted a repeated measures ANOVA to assess change in aggressiveness over time in a trial using the values of mean aggression score for each ant. Our data met all of the assumptions for the statistical analyses performed.

RESULTS
Ants from focal trees were significantly more aggressive towards ants introduced from a distant tree (mean (µ) ± 1SE = 3.86 ± 0.36) than towards ants introduced from the nearest neighbor (µ = 2.40 ± 0.29) or re-introduced from the focal tree itself (µ = 1.95 ± 0.31, ANOVA F_{2,35} = 8.50, P = 0.001; Figure 1). Post-hoc analysis of pairwise differences indicated that the most aggressive response towards the distant ants was significantly higher than towards the focal or neighboring ants. However, the most aggressive response between the focal and neighboring ants was not significantly different (Figure 1).

The mean behavior scores differed significantly among the three treatments over time (Repeated Measures ANOVA: Wilks’ Lambda = 0.67, F_{4,80} = 5.31, P = 0.008; Figure 2). Aggression towards ants from distant trees increased with time (µ = 1.30, 2.05, 2.73) while aggression towards ants from neighboring and focal trees decreased (µ = 1.30, 1.21, 1.00; µ = 1.27, 0.92, 0.47).

DISCUSSION
Our results suggest that acacia ants were able to determine whether introduced ants were familiar (neighboring or reintroduced residents) or unfamiliar. In the first minute of our trials, focal ants reacted uniformly to all introduced ants. We interpreted their behavior as non-aggressive, but inquisitive, as if they were identifying the introduced ant as friend or foe. The focal ants’ subsequent reaction indicated the relative threat level posed to the colony by the introduced ant. The finding that focal ants reacted aggressively towards distant ants and non-aggressively towards neighboring and reintroduced focal ants provides
evidence of the dear enemy effect and supports our hypothesis.

The ability to distinguish among co-occurring ants is important to the preservation of an ant colony’s territory and a crucial component of the dear enemy effect (Temeles 1994). However, the mechanism of recognition among ants is not agreed upon in the literature. Heinze et al. (1996) found that recognition among ants occurs via antennal exchange of cuticular hydrocarbons. Brandstaetter et al. (2008) observed that most ant species are able to distinguish nestmates from foreign ants at a distance up to one 1 cm. Regardless, close proximity is vital to recognition in ants, a fact consistent with our observation that *C. crinosa* interacted non-aggressively with all introduced ants at the beginning of each trial.

There are at least two ways to interpret the focal ants’ reaction to the neighboring ants. In accordance with the dear enemy hypothesis, ants must be capable of not only determining which ants are not from their home colony, but also distinguishing among foreign colonies. Alternatively, the focal ants’ non-aggressive behavior towards neighbors could result from the possibility that they are members of the same colony, as *C. crinosa* colonies are sometimes polydomous, with ants of the same colony traveling between multiple trees (Gattie et al. 2002). We suggest that the former interpretation is more likely since focal ants decreased their level of aggression more quickly towards members of their own colony than they did towards neighboring ants.

Interestingly, when expelling introduced distant ants, focal ants would often throw themselves from the tree along with the intruder. As workers are non-reproductive, they can only increase their reproductive fitness through kin selection (Queller and Strassmann 1988) as a means of contributing to the fitness of their colonies. This ostensibly altruistic act of sacrificing themselves should improve colony fitness by removing invaders, thus indirectly benefitting the evolutionary fitness of the worker (Forester et al. 2006).

The dear enemy effect in *C. crinosa* reflects the cooperation required for the ant-acacia mutualism. The ants protect their home and resources against territorial intruders by reacting defensively against strangers, yet save energy by recognizing neighbors and not reacting.
aggressively. Because thousands of ants share a single tree, the detection and expulsion of strangers requires intense cooperation. The acacia ants’ ability to quickly and accurately distinguish between neighboring and foreign ants, and react appropriately, demonstrates the highly efficient and ritualized functioning of an ant colony.

ACKNOWLEDGEMENTS
We thank Dartmouth College for sponsoring our experiment as well as the OTS research station at Palo Verde for allowing us to conduct research on their grounds.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
TWO ALARMS FOR ONE TREE?
DIFFERENTIAL RESPONSE OF *Pseudomyrmex spinicola* TO MULTIPLE STIMULI

GILLIAN A.O. BRITTON, SAMANTHA C. DOWDELL, ELIZA W. HUNTINGTON, ELLEN T. IRWIN, AND MARIA ISABEL REGINA D. FRANCISCO

Project Design: Zachariah Gezon; Faculty Editor: Ryan Calsbeek

**Abstract:** Dynamic investment optimization theory describes how organisms perform services to maximize profit by reducing costly energy expenditure. Such optimization is seen within mutualistic relationships where each party provides services in a manner which minimizes cost and maximizes benefit. In the mutualism between red acacia ants (*Pseudomyrmex spinicola*) and acacia trees (*Acacia collinsii*), *P. spinicola* defend a host acacia tree by responding to both physical and chemical stimuli, which may indicate herbivore activity. Recruitment of ants to the site of disturbance is energetically costly, and thus response to “false alarms” should be minimized. We predicted that *P. spinicola* would have a greater response to physical stimuli than chemical stimuli, as physical stimuli occurs both before and during herbivory and is thus a more immediate indication of herbivore presence. We also expected to see a non-additive response to the combination of the two types of disturbance because both cues together are more likely to indicate true herbivore activity. We measured ant recruitment on 28 *A. collinsii* trees in response to four experimental treatments: physical disturbance, chemical disturbance, physical + chemical disturbance, and no disturbance (control). While ants showed recruitment after physical disturbances, we found no response to chemical stimuli and no physical*chemical interaction. Our results demonstrate that physical disturbance is the primary alarm leading to initial ant defense. While further study is necessary to confirm the role of multiple stimuli, our observations suggest that chemical stimuli may play, at most, a secondary role resulting in a prolonged defense response, and that the cost of false alarms may be sufficiently small as to make a multiple alarm system unnecessary.

**Key words:** defense mechanisms, dynamic investment optimization theory, mutualism, *Pseudomyrmex spinicola*

**INTRODUCTION**

Mutualisms, species interactions which benefit both parties involved, are ubiquitous throughout nature. For a mutualism to function, both species must provide a service to the other at a cost lower than the benefit of a reciprocal service. Dynamic investment optimization theory describes the manner in which organisms seek to minimize cost and maximize gain (Bronstein 2001, Roughgarden 1975). Such optimization is apparent within mutualistic relationships: it is costly for such organisms to provide services for their partners (Bronstein 2001). Therefore, to benefit from the interspecies interaction, a mutualist must minimize energy expenditure while continuing to uphold their side of the bargain.

In exchange for food and shelter, red acacia ants, *Pseudomyrmex spinicola* (Hymenoptera, Formicidae, Pseudomyrmecinae), defend host acacia trees, *Acacia collinsii* (Fabaceae), by attacking active herbivores (Romero and Izzo 2004). The ants respond to both physical and chemical stimuli that may indicate herbivory, such as branch movement (Janzen 1966) and the release of volatile leaf compounds (Agrawal 1988). Little work has been done, however, on the differential response of acacia ants to physical versus chemical stimuli and the combined effect of both stimuli. Huntzing et al. (2004) showed that trees that are experimentally protected from herbivory reduce production of ant-rewards, as it is costly to invest in ant nourishment. It is also energetically costly for acacia ants to respond to stimuli as this involves recruitment to the location of disturbance (Jensen and Holm-Jensen 1980). Therefore, responses to “false alarms,” or those that are not the result of herbivore activity, should be minimized in order to maximize the benefit of the mutualism.

The purpose of this study is to determine if *P. spinicola* differentiate between physical, chemical, and combined stimuli. We predicted that *P. spinicola* would mount a greater response to physical stimuli than chemical stimuli, as physical stimuli occur both before and during herbivory and are thus a more immediate indication of herbivore presence. There is a probability that both physical and chemical stimuli indicate false alarms, and thus responding to the stimuli is wasted energy. If the stimuli are independent of...
one another however, the probability of the simultaneous stimuli indicating a false would be the product of individual probabilities, and thus dramatically lower. We therefore predicted that there would be a non-additive response to a combination of physical and chemical stimuli because both cues together are more likely to indicate true herbivore activity. The presence of a double alarm system could help explain the efficiency of the *A. collinsii* defense mechanism and thus the success of the acacia-ant mutualism.

**METHODS**

We conducted our experiment on January 11, 2013 in Palo Verde National Park, Guanacaste, Costa Rica. We haphazardly selected 28 acacia trees (*A. collinsii*) that hosted colonies of red acacia ants (*P. spinicola*) and with trunk diameters of approximately 3cm. We excluded trees that were in direct contact with a previously tested tree or that were disturbed by accidental physical disturbance. We randomly assigned each tree to one of four treatments (chemical, physical, chemical+physical, or no disturbance (control)) by drawing pre-labeled strips of paper out of a hat. We tested physical disturbance by hitting the trunk of the tree three consecutive times at a height of approximately 1.5m (a height that made observations easy without inadvertent physical disturbance to the tree). We tested chemical disturbance by selecting a branch of the same tree approximately 1 m off the ground and carefully cutting off the leaf at the end of the branch, ensuring that the branch was not jostled in the process. We then smeared the leaf directly onto the tree trunk at a height of approximately 1.5m. In the combined treatment, we performed the chemical treatment first and immediately followed it with the physical disturbance, applied at the same point on the tree trunk. Immediately after treatment, we measured ant recruitment for two minutes as the number of ants passing the thorn nearest to the disturbance. We counted ants moving both up and down the tree past the designated observation thorn. Individual ants were not monitored; every passing was counted regardless of whether the same ant was repeatedly passing the thorn. Two counters stood on opposite sides of the thorn and counted every passing ant in their visual field using clickers. After data collection, the ant counts from the two observers were averaged. We repeated this methodology for the control treatment, excluding the application of any physical or chemical stimuli.

**Statistical Analysis**

We checked the univarite distributions for normality and outliers before performing statistical analyses. We used a two-way ANOVA to test the interaction of physical and chemical disturbance. We confirmed normally distributed residuals using the normal quantile plot, and equal variances of residuals by the Levene’s test. Finally, non-significant factors were removed from the final model: an ANOVA comparing physical versus no physical stimuli on the recruitment of acacia ants. All assumptions for the final model were confirmed using the aforementioned techniques. We performed all analyses using JMP 10.0.

**RESULTS**

Using a two-way ANOVA we found no significant interaction between physical and chemical disturbance ($F_{1,24}=0.29, P=0.59$; Figure 1) on *P. spinicola* recruitment. The main effect of physical disturbance was highly significant ($F_{1,24}=11.22, P=0.003$; Figure 1), but we found no significant
main effect of chemical stimuli ($F_{1,24}=0.12$, $P=0.73$; Figure 1). When chemical disturbance was removed from the final model, we found a more highly significant effect of physical stimuli on recruitment ($F_{1,26}=11.95$, $P=0.0019$). The Levene’s test assured that the assumption of equal variance of residuals needed for a two-way ANOVA was met ($F_{3,24}=1.89$, $P=0.16$).

**DISCUSSION**

As we predicted, physical stimuli induced higher ant recruitment as compared to the no-physical treatments. However, we found no significant effect of chemical stimuli nor did we find a non-additive effect of chemical and physical stimuli combined. In keeping with the dynamic investment optimization theory, it appears that ants are able to differentiate among stimuli, which may enable them to reduce energy costs associated with recruiting ants to false alarms. Our results suggest that physical stimuli are the main cues initiating the defense response of *P. spinicola* to herbivory on *A. collinsii*. Ants did not appear to utilize chemical stimuli or the combination of stimuli to determine herbivore activity, as chemical cues may have a higher probability of raising a false alarm or may be too weak a signal to induce a defensive response from the ants.

Previous studies have shown that both physical and chemical signals induce acacia ant recruitment (Agrawal 1988). Our results demonstrate that physical disturbance is the primary alarm leading to initial ant defense. While further study is necessary to confirm the role of chemical stimuli, our observations suggest that chemical stimuli may play, at most, a secondary role resulting in a prolonged defense response. Agrawal and Rutter (1998) observed that ant recruitment to damaged leaves increased 400% within four minutes and continued for several hours, indicating that ants respond to a combination of physical and chemical stimuli within the *Cecropia-Azteca* ant-plant mutualism. Based on these findings and our observations it is possible that while physical stimuli are an indication of initial herbivore presence, chemical stimuli may confirm herbivore activity leading to further defensive action. Therefore, had observation time been extended, results may have indicated a role of both a primary physical and a secondary chemical alarm system.

While further study is necessary to explore the existence of a secondary alarm system triggered by chemical cues, the primary alarm system, which induces rapid response to physical disturbance, helps explain the efficiency with which acacia ants defend against herbivore presence. What’s more, the lack of an interaction between physical and chemical stimuli indicates that the cost of false alarms may be sufficiently small as to make a multiple alarm system unnecessary. The ability of acacia ants to optimize defensive responses and minimize energy expenditures by responding primarily to physical stimuli contributes to the success of the mutualism between *P. spinicola* and *A. collinsii*. Understanding this success sheds light on the necessary balance between mutualism expenditure and individual optimization.

**ACKNOWLEDGEMENTS**

We would like to thank the staff of Palo Verde National Park for providing transportation and sustenance, and Zachariah Gezon for his assistance in experimental design and general guidance and support.

**AUTHOR CONTRIBUTIONS**

All authors contributed equally.

**LITERATURE CITED**


Palo Verde


Survivorship and Resistance in the Predator-Prey Interactions Between Ants and Antlions

Amelia F. Antrim, Tyler E. Billipp, Maria Isabel Regina D. Francisco, Elisabeth R. Seyfert, and Victoria D. Stein

Project design: Ramsa Chaves-Ulloa; Faculty Editor: Ryan Calsbeek

Abstract: Predator-prey interactions impose selection pressures that affect the relative success and failure of both populations, particularly for prey, since their stakes are higher in each encounter. These predator-induced pressures do not necessarily act evenly across related prey taxa. Increased exposure to predators often strengthens the selection pressure that drives the evolution of predator avoidance mechanisms. We predicted that, because terrestrial ants share a foraging environment with antlion larvae (Myrmeleontidae spp), they would have higher antlion-pit escape ability than arboreal ants. We tested three species each of ants found foraging in terrestrial and arboreal habitats by placing them in antlion pits. We found no significant difference in antlion escape ability between terrestrial and arboreal ants. However, terrestrial Ponerine ants (the species most often observed near antlion pits in the field) resisted antlions much longer than all other species and were the only ants to escape the pits. Resistance time also increased significantly with ant body size across species. Ponerine ants had the largest mean body size of the six species, which may have been a key factor in their escape capability. Our results suggest support for predator-induced adaptation driven by differential predation pressure on populations of prey.

Keywords: antlions, escape mechanisms, predator-prey interactions

Introduction

Selection pressures imposed by predator-prey interactions affect the relative success and failure of each population. Repeated interactions improve predator resistance in prey and refine capture mechanisms in predators (Abrams 2000). Since the stakes are much higher for prey than predator, selection pressures are usually asymmetrical, driving more rapid evolution in prey (Dawkins and Krebs 1979). Predation pressures also do not necessarily act evenly across related prey taxa, imposing stronger pressures on populations that are most heavily exposed to particular predators (Snell et al. 1988, Vervust et al. 2007). Varying exposure to predators might therefore dictate the strength of the predation pressure acting on prey.

Antlion larvae (Myrmeleontidae spp.) are terrestrial predators that prey on ants and other small ground-dwelling insects by building circular pitfall traps in fine dirt (Coelho 2001). Small insects often cannot escape from antlion pits because the walls assume the angle of repose of the soil and disturbances cause the pit sides to slide downward (Coelho 2001). To avoid death, ants that fall into the pit must avoid the antlion’s mandibles or escape their grasp and then ascend the pit walls. Because of their efficient and highly specialized prey capture method, antlions potentially exert strong selective pressure on their prey.

Terrestrial ants interact with antlions more frequently than do arboreal ants because of their shared habitat; therefore, our study tested the prediction that terrestrial ant species would be better adapted than arboreal ants to delay or avoid death when introduced to an antlion pit.
METHODS

This study was conducted on January 12th and 13th, 2013 in Palo Verde National Park, Guanacaste, Costa Rica. We collected 30 antlions from roadside soil and transferred the antlions individually to plastic cups containing two inches coarse sand and one inch fine roadside soil. We left each cup indoors for at least two hours and ensured that each antlion had formed its trap before conducting the experiment. We also collected six species of ants, three of which (subfamilies Ponerinae, Dolichoderinae, and Myrmicinae) were found foraging on the ground and three of which (Pseudomyrmex flavicornis, P. ferruginea, and Crematogaster crinosa) were primarily arboreal mutualists with Acacia collinsii and A. cornigera, deriving most of their food and shelter from those trees. For each species, we collected ants from five separate colonies, for a total of 30 ants.

On the day of the ants’ capture, we used an online random number generator (random.org) to randomize the testing order of ant species, which colony we tested per species, and which antlion we used for each trial. Using forceps, we dropped the ant into the center of the pit and observed the ant and antlion for four minutes, recording time of first observed antlion attack and either time of death or time of escape. For the ants that died, we calculated “resistance time” as the time of death minus the time of first attack. We then tested ants from the five remaining species and repeated this process until we had tested ants from the five colonies for each of the six species. We used one ant per antlion, using 30 antlions and 30 ants in total.

To account for size effects in our treatments we measured the length of each ant and antlion from the insects’ head to abdomen, not including mandibles. We then calculated the mean ant body size for each of the six ant species. We also measured the diameter of the pits built by the antlions. All measurements were performed using dial calipers.

To test for the differences in ant survival as a function of foraging habitat (terrestrial versus arboreal), we used a right-censored survival analyses. We then performed a similar analysis with Ponerine ants versus all other ants as the explanatory variable. To account for the repeated analyses we used a Bonferroni corrected α value of 0.025. To test if ant body size varied significantly as a function of species, we used ANOVA with Tukey’s HSD post hoc comparisons. We also performed a bivariate fit of ant body size against resistance time, assigning a resistance time of 240 seconds (4 minutes) to ants that survived. All data fulfilled the assumptions of the corresponding statistical tests. We used JMP 10.0 (SAS Institute, Inc. 2012) for all statistical tests.

RESULTS

Resistance time did not differ between arboreal and terrestrial ants ($\chi^2=0.42$, df=1, $P=0.51$; Fig. 1). Arboreal ants struggled for an average of 124.79 seconds (SE=18.44), while terrestrial ants struggled for an average of 130.00 seconds (SE=19.57).

![Figure 1. We found no significant difference in ant resistance time to antlion attacks between terrestrial and arboreal ants.](image-url)
However, a second right-censored survival analysis revealed a significant difference between resistance time of Ponerine ants as compared to all other ant species even after Bonferroni correction of the alpha value to 0.025 ($\chi^2=9.57$, df=1, $P=0.002$; Fig. 2).

The mean resistance time of Ponerine ants was 212.60 seconds (SE=32.13 seconds), while the mean resistance time of all other ants was 110.33 seconds (SE=12.39 seconds). Mean ant body size had the highest mean ant body size out of all six species, but the difference was not significant (Fig. 4). We also found that resistance time time increased significantly and linearly with ant size across all species ($\chi^2=0.20$, $P=0.02$; Fig. 3).

**DISCUSSION**

We found little support for any advantage in antlion escape ability in terrestrial over arboreal ants. When compared against all other species, however, terrestrial ants of the subfamily Ponerinae exhibited significantly higher survivorship in terms of resistance time and probability of escape. Ponerine ants were the only group to escape the antlion pits, doing so in three of five (60%) trials. Interestingly, these ants were the primary ant species observed foraging around the antlion pits. This observed proximity suggests that Ponerines may encounter antlions most regularly and that antlion predation pressure may act more strongly on Ponerine ants than on other species tested, although causality is not clear. Ponerines might forage near antlions because of their ability to escape the pits.

Our results also show that body size plays a major role in antlion resistance and probability of pit escape. The positive relationship between ant body size and resistance time, which accounted for nearly 20 percent of the variation in resistance time (Fig. 3), indicates that relatively large body size may have been a key factor in Ponerine ant survival. Although the difference was not
statistically significant, Ponerine ants had the largest mean body size of the six trial species (Fig. 4). When predator and prey differ only slightly in relative size, changes in prey body length may have large effects on survival in predator encounters. Thus, antlion predation may select for larger body size in ants in the same way predation has been shown to drive size increase in other organisms (Losos et al. 2004; Vervust et al. 2007). However, ant size and morphology may be influenced by other selection pressures such as metabolic demands or sexual selection (Chown and Gaston 2009).

Further studies could continue to investigate this dynamic by comparing resistance abilities of similarly-sized ants evolved either in the presence or absence of antlions. Additionally, more work could be done regarding other Ponerine adaptations against antlion predation, since their escape abilities do not seem to be explained by body size alone. Recognizing how predator-induced pressures influence different populations of prey can inform our understanding of morphological and behavioral adaptation.

ACKNOWLEDGEMENTS

We would like to thank the staff of Palo Verde Biological Research Station for their support, Ramsa Chaves-Ulloa for inspiring the experimental design, and Professor Ryan Calsbeek and Zak Gezon for their feedback and assistance.

AUTHOR CONTRIBUTIONS

All authors contributed equally to experimental design, execution of experiment, and writing of the manuscript.

LITERATURE CITED


INVESTIGATING THE EFFECTS OF APOSEMATISM ON PREDATOR AVOIDANCE

SETH A. BROWN, SAMANTHA C. DOWDELL, ELIZA W. HUNTINGTON, ELLEN T. IRWIN, AND KALI M. PRUSS

Faculty Editor: Ryan Calsbeek

Abstract: Many toxic animals have evolved bright coloration patterns to warn, and thus avoid, potential predators. To test the effectiveness of aposematism on predation, we designed aposematic (red, yellow, black) and non-aposematic (green) model snakes using clay and colored paints, and set them out in a forest clearing. We found that green snakes were attacked more frequently (though not significantly) than aposematic snakes, which supported both our hypothesis and results from previous research. Our results demonstrate that aposematism provides protection from predators, as predators tend to avoid brightly colored prey.

Key words: Aposematism, coral snake, predator defense

INTRODUCTION
Organisms have evolved a variety of defense mechanisms to protect themselves from predators. For example, many prey invest in the production of toxins to make themselves unpalatable (Broom et al. 2008). However, distastefulness alone may provide insufficient protection, as predators would not know to avoid toxic prey except through individual experience (Harvey et al. 1981). For this reason, prey organisms often use auditory, olfactory, and/or visual signals to advertise their toxicity (Ham et al. 2006). These warnings, known as aposematism, are an effective defense because some predators have evolved an innate aversion to such signals (Brodie and Janzen 1995). Aposematism is a well-studied phenomenon in nature (Wuster et al. 2004) and has been observed in many insect species, mammals, and snakes.

One of the most well-known cases of aposematism occurs in the coral snake. To advertise their high toxicity, coral snakes have bright warning colors in a distinct banded pattern (Smith 1975). Many predators have an innate aversion to these markings (Brodie and Janzen 1995). Previous studies have found that birds avoid all similar banded patterns in clay snake models, regardless of whether or not the pattern specifically mimics a local toxic-snake (Brodie 1993; Brodie and Janzen 1995).

METHODS
To test predator avoidance of aposematic coloration we constructed 20cm long clay snake models. We used tempera paints and painted nine snake models to match the color patterns (red, yellow, and black bands) of Micrurus nigrocinctus, a local coral snake, and we painted nine additional snake models as generic snakes. Generic snakes were painted dark green with a white diamond pattern to resemble locally-occurring non-venomous snakes. We used a partially wooded field in Palo Verde National Park, Costa Rica as our study site. We randomly assigned each snake to a 5m x 5m square in a 20m x 30m grid in the field and placed each model approximately in the center of its assigned square. We left the models out from 3:00 PM until 7:00 AM. At the end of the experiment, we collected all clay models and recorded whether or not each had been attacked.

Statistical Analysis
All statistical analyses were performed using JMP v. 10.0. We used a chi square test on the proportion of snakes attacked for each treatment. We performed a post-hoc power analysis using the statistical program R, to determine the necessary sample size to find significant results in cases where our experimental results were not
significant. Our data met the assumptions for all statistical analyses.

RESULTS
We found that 67% of the green snakes were attacked, while only 38% of the aposematic snakes were attacked (Figure 1). Although the pattern matched the direction predicted by our hypothesis, this difference was not significant ($\chi^2=1.466$, df=1, p=0.226). A power analysis revealed that a sample size of 149 would be needed to yield significant results.

DISCUSSION
The pattern demonstrated by our data supports our hypothesis that green snakes would be attacked more frequently than aposematic snakes. Although we failed to reject the null hypothesis, our results are consistent with the hypothesis that aposematism provides protection from predators. Since none of our models varied in palatability, predators avoided brightly colored snakes by discretion alone. Additionally, results from our study are consistent with the work of Brodie and Janzen (1995), who performed a similar experiment in Palo Verde and found that avian predators exclusively attacked the non-aposematic models.

We encountered various difficulties during experimentation that may have detracted from the trend we predicted. We originally set out a total of 36 model snakes that were divided evenly between treatments and between two sites. However, we were forced to discard one site from our study due to model damage inflicted by human foot traffic, cars, and a tractor. Consequently, our sample size was severely decreased, making our results less robust. According to our power analysis, if we had been able to increase our sample size considerably we would have produced significant results. Thus we suggest that our data support a real biological phenomenon.

In the study by Brodie and Janzen (1995), also completed in the forest of Palo Verde, no aposematic models were attacked. As our study was conducted in a clearing, it would be interesting to compare attack rates on aposematic snakes between forest and field. Because coral snakes are more commonly found in the forest (Brattstrom 1955), perhaps birds that hunt in the forest have a stronger aversion to coral snake patterns than those that hunt in more open areas. In addition, there is evidence that gregariousness in aposematic species results in decreased predation. Several studies (Mappes and Alatalo 1997; Lindstrom et al. 1999) have found that aggregated aposematic organisms have higher survival, potentially because of the heightened stimulus produced by aposematic groups. Since aposematic species must be easily recognizable to a predator after first encounter (Harvey et al. 1981), we suggest future studies should test whether a minimum threshold in signal strength is necessary for predator deterrence. We speculate that large coral snakes can survive alone whereas small aposematic organisms, such as insects, must group together to create a large-enough warning signal. Such a study could demonstrate a limit to the benefit of group behavior may exist when an individual already has effective defense mechanisms, shedding further light on the concept of aposematism (Gamberale and Tullberg 1996).

ACKNOWLEDGEMENTS
We would like to thank Ryan Calsbeek for his thoughtful question and insightful background knowledge, as well as Ramsa Chaves-Ulloa and Zachariah Gezon for their assistance with the statistical analyses.

AUTHOR CONTRIBUTIONS
All authors contributed equally to this paper.

LITERATURE CITED


A PRODUCER-CONSUMER RELATIONSHIP: NECTAR ADVERTISING IN EICCHORNIA CRASSIPES

GILLIAN A. O. BRITTON, COLLEEN P. COWDERY, JIMENA DIAZ, EMILIA H. HULL, MOLLY R. PUGH

Project Design: Zachariah Gezon; Faculty Editor: Ryan Calsbeek

Abstract: Pollinator mediated selection has resulted in a variety of sizes, colors, and scents of floral displays as angiosperms compete for pollinators. Some studies have suggested that ultraviolet (UV) nectar guides are an example of pollinator-mediated adaptations. Many angiosperms exploit insect visual sensitivity by using UV nectar guides to attract and orient potential pollinators. We explored the role of UV nectar guides on water hyacinth (Eicchornia crassipes) in attracting honeybees (Apis mellifera) in Palo Verde National Park, Costa Rica. Previous studies have shown that nectar guides increase plant reproductive success by attracting more pollinators. We tested the general hypothesis that the presence of UV nectar guides increases pollinator visits and pollen receipt. We predicted that the presence of UV nectar guides on water hyacinth would attract more pollinators, and that experimentally obscuring nectar guides would decrease the number of honeybee visitations and pollen receipt. We found that the presence of a nectar guide did not act to attract honeybees to a plant, but that the average number of flowers that a honeybee visited per plant was higher when nectar guides were un-obscured. We concluded that UV nectar guides are short-range signals that serve to increase the number of flowers per stalk that an individual bee visits. The implications of this study raise questions as to whether nectar guides are ecologically beneficial or costly, as they appear to be a poor long-distance advertisement of a plant’s nectar source, but work to keep honeybees on the plant once they have arrived.

Key words: Apis mellifera, Eicchornia crassipes, nectar guide

INTRODUCTION
The vast majority of flowering plants rely on pollinators for reproduction (Gurevitch et al. 2002). While many plants are capable of self-pollination, pollinators increase genetic variation among offspring by cross-pollination, often resulting in overall higher fitness (Ellestrand and Elam 1993; Barrett 1980). Thus, there may be strong selective pressure for floral morphology that attracts pollinators (Medel et al. 2003). Nectar guides are one such adaption of angiosperms. Nectar guides can have both olfactory and visual properties that are attractive to bees (Free 1970). Previous studies have suggested that nectar guides are used to advertise a plant’s nectar source, increasing its attractiveness to pollinators (Medel et al. 2003; Waser and Price 1985) and its overall plant fitness and reproductive success (Hansen et al. 2012).

There remains limited work done on the role of nectar guides in pollination biology, perhaps due to the difficulty of field experiments. We investigated the role of ultraviolet (UV) nectar guides in the mutualism between the invasive water hyacinth Eicchornia crassipes (Pontederiaceae) and Africanized honeybee Apis mellifera scutellata, (Hymenoptera, Apidae). We hypothesized that the presence of UV nectar guides on water hyacinth would increase the number of honeybee visitations and pollen receipt compared to those plants with experimentally obscured nectar guides. Increased visitation and a higher pollen receipt would illustrate the benefit of investing in nectar guides as a way to increase both male and female reproductive fitness. Understanding the adaptive significance of nectar guides will shed light on the selective pressure pollinators place on angiosperms and the evolutionary race to improve floral attractiveness to pollinators.

METHODS
We conducted our study on January 13 and 14, 2013, in the Palo Verde marsh, at Palo Verde National Park, Guanacaste, Costa Rica. Six observers each haphazardly selected four E. crassipes inflorescences for a total of 24 inflorescences. Each group of four inflorescences was within a 1m-radius circular plot, enabling observation of all inflorescences simultaneously. Three observers chose
inflorescences near the shore and three observers chose inflorescences in open marsh. Each plant was randomly assigned to one of two treatments (treatment or control) by drawing pre-labeled strips of paper out of a hat. Experimental flowers had their nectar guides obscured with a thin layer of Coppertone™ oil-free, fragrance-free sunscreen applied with a small paintbrush. Control flowers had the same application of sunscreen to the petal to the right of their nectar guide to control for the effect of applying sunscreen.

In the early morning, prior to the flowers’ opening, we recorded the number of flowers per inflorescence on each of our focal plants. We applied the sunscreen when flowers first opened (ca. 7:30 am). We performed pollinator observations during peak foraging time (7:45 am to 9:00 am). We recorded the total number of honeybee and other bee visits to each inflorescence, as well as the number of flowers visited per inflorescence by each bee (hereafter referred to as “repeated visits”). A “visit” was defined as a honeybee landing on an individual flower of an inflorescence. Bees were not tracked after leaving an inflorescence and a bee was counted as a new individual if it left the inflorescence and returned later. Following pollinator observations, we noted the density of plants in each 1m-radius circular plot. We then removed the stigma from focal flowers using tweezers. Stigmas were refrigerated until microscope slides were prepared using basic fuchsin jelly (Kearns and Inouye, 1993). We counted the number of pollen grains per stigma (hereafter referred to as “pollen count”) using a compound microscope at 40X.

Statistical Analysis
We used JMP 10.0 statistical software for all analyses. We analyzed the univariate distribution of our response variables, including number of visits, number of repeated visits per plant, and pollen count per stigma. We tested for normality of all variables using normal quantile plots. To test for differences in pollen receipt between treatments we used two sample t-test. We also compared the repeated number of visits to a plant per bee with treatment using a two sample t-test. Additionally, we performed two regression analyses on the number of pollen grains per stigma as a function of number of flowers per stalk, and on honeybee visits as a function of flowers per stalk.

RESULTS
We found no differences in mean pollen grains per stigma between control and treatment inflorescences (mean pollen grains per stigma ± 1SE: µcontrol = 10.75 ± 3.29, µtreatment = 10.64 ± 2.76 pollen, t22 = 0.09, P = 0.93; Figure 1).

Though the nectar guides did not affect mean number of honeybee visits to an inflorescence, there was a significant difference between treatments in the mean number of repeated visits (µcontrol = 0.49 ± 0.06 visits and µtreatment = 0.06 ± 0.58 visits, t22 = 2.40, P = 0.03; Figure 2). We found that the pollen count increased with an increasing numbers of flowers on an inflorescence (r² = 0.19, P = 0.03, Figure 3). We also found that the number of honeybee visits increased linearly with the number of flowers per inflorescence (r² = 0.19, P = 0.001, Figure 4).
DISCUSSION

Our results suggest that the number of flowers per inflorescence was the most significant factor affecting the number of honeybee visits and pollen receipt. Contrary to our hypothesis, UV nectar guides do not appear to play a role in attracting bees to an inflorescence. However, our results demonstrate that the presence of nectar guides increases the number of repeated visits, which could act positively or negatively on the plant’s overall reproduction. Honeybee visitation to multiple flowers increases male reproductive fitness as it increases pollen export (Sutherland and Delph 1984). However, female fitness and overall water hyacinth reproductive success benefit from cross-pollination (Gurevitch et al. 2002, Barrett, S.C.H. 1980). Therefore if a bee brings pollen from other plants, the receipt of pollen on multiple flowers is beneficial to the plant’s reproduction (Lang and Danka 1991). However, if bees only serve to move the plant’s own pollen between different flowers on the same inflorescence, no cross-pollination is achieved and the plant may be primarily self-pollinating. Our findings suggest that nectar guides may increase male reproductive fitness, but further study is necessary to determine effect on female fitness.
Other studies have found that nectar guides use multiple types of cues to increase reproductive success (Free 1970). Flower scent has been shown to have a stronger effect on honeybee foraging preferences than attributes such as coloration or flower size (Free 1970). Thus, it is possible that the bees were unaffected by the covering of UV guides because they were able to detect the flowers by other cues such as scent or non-UV related visual cues. Additionally, our handling of the plants or the chemicals in the sunscreen may have damaged the flowers, making them less appealing to the bees than they would have been otherwise. We were unable to determine differences from natural visitation behavior as our experiment did not contain a true control; instead, we opted for a sham control. We believed the sham was more useful than a true control, as it controlled for all effects of sunscreen, other than UV obscurance, equally among all flowers (Johnson and Andersson, 2003).

Our study sheds light on the long-range effectiveness of inflorescence flower numbers and the short-range effectiveness of UV nectar guides in attracting honeybees. Further research is necessary to fully understand the spatial scale on which nectar guides work. For example, the collective effect of UV nectar guides in an entire patch of water hyacinth may serve to attract more honeybees to that patch than to a patch without such guides.

ACKNOWLEDGEMENTS
We would like to thank the staff of Palo Verde National Park for providing transportation and sustenance, Z. Gezon for his assistance in experimental design and general guidance and support, and R. Chaves-Ulloa and R. Calsbeek for their feedback and assistance.

AUTHOR CONTRIBUTIONS
All authors contributed equally

LITERATURE CITED
PREDATOR AND ALARM CALL RESPONSE IN CAPUCHIN MONKEYS

AMELIA F. ANTRIM AND KALI M. PRUSS

Abstract: Predator recognition and communication of threat-information are important components of predator avoidance in social animals. Many animals use visual, olfactory, and auditory cues to assess predation risk and make subsequent behavioral decisions, which are often acquired through conditioning. Few studies have investigated the difference in behavioral response to an alarm call versus the vocalization of a predatory animal itself, or whether primates can learn to interpret alarm calls of other species. We tested the behavioral response of Cebus capucinus to four different calls: an avian predator (Swainson’s hawk), a terrestrial predator (jaguar), a conspecific alarm call (capuchin), and the alarm call of a common co-occurring primate (howler monkey). We tested two hypotheses: 1) Capuchin monkeys will respond more to their own alarm than all other calls; 2) capuchins will respond more to their own alarm call than another primate’s alarm call. We used time spent “searching” as an estimate of individual response time, and found no significant difference in response across the four treatments. However, capuchins spent more time searching and performing aggressive displays in response to a conspecific alarm call than the other 3 treatments combined. We suggest that the capuchins treated the conspecific alarm as an extra-troop alarm call, and therefore responded aggressively.

Keywords: alarm call, Cebus capucinus, predator response

INTRODUCTION

Heightened predation pressure has been known to increase cooperative behaviors in some organisms (Krams 2009). Thus, predation responses are often augmented in social animals who warn each other about potential dangers; anti-predator vigilance is an important advantage of group living (Hirsch 2002). However, to maximize the anti-predator benefits of gregariousness, organisms must be able to communicate threats to group members. A common communication method is alarm calling, a behavior that has been observed in gregarious primates. Primates emit vocalizations that elicited uniquely in response to a predator, warning their group of the specific threat (Seyfarth 1980).

Social learning shapes white-faced capuchin (Cebus capucinus) alarm calls and responses. Juvenile monkeys often emit “false” alarm calls in response to non-predatory organisms and immature capuchins hone their alarm-call accuracy by observing adults in the troop (Perry 2003), suggesting that appropriate responses to different predators are learned through conditioning (Brown 1998). Because capuchins learn about predator response through experience, responses and alarm calls may vary between troops.

Many studies have explored capuchin responses to conspecific alarm calls (Fichtel et. al 2005, Digweed et. al 2005, Digweed et. al 2007), but few have investigated differences in behavioral response to an alarm call versus the vocalization of a predatory animal itself. To explore this, we observed capuchin behavior in response to four different calls: two types of predatory calls and two different alarm calls. Since capuchins are most conditioned to respond to a call from a member of their own troop, we hypothesized that capuchins would respond more to the conspecific alarm call than any of the other three calls.

Capuchins also respond to alarm calls emitted from conspecific members of other troops (Wheeler 2009). As a secondary focus on our study, we investigate the more specific question of whether capuchins have developed the ability to respond to alarm calls of another co-occurring primate. This question has not, to our knowledge, been previously investigated. If capuchins could become aware of the presence of a predator without a member of the troop encountering it, they would increase their likelihood of survival. We observed capuchin response after hearing their own alarm call and an alarm call of the mantled howler monkey (Allouatta paliatta); we expected the capuchins
to have a greater response to their own alarm call.

METHODS
We observed capuchin response to four different calls of local animals: Swainson’s hawk (*Buteo swainsoni*), jaguar (*Panthera onca*), conspecific alarm call (capuchin), and the alarm call of a common co-occurring primate (howler monkey). We used calls of both a terrestrial and avian predator to account for varying responses to different predator types. Predators were chosen based on previous studies and consultation with naturalists in the area. The alarm calls of both primate species were emitted in response to a predator encounter. We conducted our study on January 15 and 16, 2013 in the tropical dry forest of Palo Verde National Park, Costa Rica.

After encountering a troop, we positioned ourselves as close to the middle of the troop as possible. We allowed at least 10 minutes for the troop to become accustomed to our presence, which we judged by resumption of foraging and grooming. For each trial, each of two observers haphazardly chose one individual within 20m of the speakers. We observed the baseline activity of the focal individuals for one minute before playing the call and again one minute after starting the call. We recorded the amount of time the focal individuals spent performing various behaviors, and used search time as a proxy for strength of response to the call. We randomized treatment by shuffling a playlist using an iPod nano (Apple, Inc.) and used small, portable speakers (Sonpre Mini Portable Capsule Speaker System) to amplify the call. After the end of a trial, we waited until the focal individuals returned to their baseline behavior before starting the subsequent trial on new individuals. Although we collected data on as many individuals as possible, we had to observe some individuals in a troop more than once due to the difficulties of following troops through the forest.

Statistical Analyses and Modeling
We used ANOVA to test for differences in search time between the four call treatments after normalizing the distribution of search times with a log10 transformation. To determine whether observed individuals had a greater search time response towards the capuchin alarm call than the other three calls, we used a generalized linear model with a Poisson error distribution. To determine whether individuals were more aggressive in response to the capuchin call than the other calls, we conducted an t-test with unequal variances. We used JMP 10.0 statistical software and the assumptions for all analyses were met.

RESULTS
We found no significant difference in time spent searching between the four treatments (ANOVA $F_{3,63} = 1.185, P = 0.3232$). However, when all non-capuchin calls were combined, we found that troops spent nearly twice as long searching after the conspecific call was played ($24.5 \pm 5.88$s) than after heterospecific calls ($13.56 \pm 2.46$s; Figure 1), although the difference was not significant (ANOVA $F_{1,65} = 2.94, P = 0.09$).

Figure 1. Capuchins spent 1.81 times longer searching after hearing a capuchin alarm call compared to the other three calls combined.

Likewise, the capuchins spent more time performing aggressive displays after a conspecific alarm call was played ($5.9 \pm 1.74$s) versus heterospecific calls ($0.35 \pm 0.73$s). The difference was not significant ($t_{65} = -2.94, P = 0.22$; Figure 2).
Figure 2. After hearing a conspecific alarm call, capuchins spent an average of 16.85 times longer performing aggressive behaviors than in response to the other calls combined.

**DISCUSSION**

Capuchins responded more strongly to conspecific alarm calls than any other calls used in the experiment (Figure 1, Figure 2). Most monkeys had little or no response to howler alarm calls or either of the predatory calls. Some capuchins searched for the source of the calls, but most ignored them. Contrary to our expectations, we found no evidence of different responses among the three non-capuchin calls.

The capuchins’ minimal response to howler alarm calls could be explained in multiple ways. Either capuchins cannot interpret howler alarm calls, or capuchins can interpret these calls and chose to ignore them. If the latter is true, troops must have determined the call was unthreatening and did not require an aggressive response. This could be the case, since other studies have determined that white-faced capuchins and mantled howlers utilize different niches (Tomblin and Cranford 1994) and that competition between the two is unlikely (Chapman 1987). Because capuchins and howler monkeys frequently come into contact in Palo Verde National Park, we believe it to be more likely that capuchins could be familiar with the meaning of howler alarm calls, but do not consider howler monkeys to be a threat.

Despite our expectation of a strong response to capuchin calls, we were surprised that some capuchins exhibited an aggressive response to their own alarm call; we expected the alarm call to elicit fearful behavior. Individuals who responded aggressively to the conspecific alarm call initially searched for the source of the call before baring their teeth or breaking off and shaking branches. We believe that, contrary to our hypothesis, the capuchins responded to the conspecific alarm call as if it originated from another troop, rather than a member of their own troop. The aggressive response may reflect a behavioral reaction to perceived competition. Moreover, other studies indicate that some white-faced capuchins have been observed exploiting other troops’ ability to comprehend their alarm calls to deceive competing troops into abandoning food-rich areas (Wheeler 2009). Thus, capuchin troops may gain a competitive advantage over other troops by responding to extra-troop alarm calls with suspicion.

The capuchins’ strong response to conspecific alarm calls exemplifies the communication skills that are integral to primate sociality. Capuchins have evolved a refined system of alerting their troop to the presence of a threat. Alarm calls facilitate responses appropriate to varying circumstances, and behaviors such as territory defense require unified behavior within a troop. Since predation pressure on capuchins is particularly low at Palo Verde (Rose 2003) and because capuchins utilize deceptive alarm calls, we believe that the capuchins at Palo Verde may be conditioned to respond aggressively to extra-troop alarm calls. Primate behavior is highly adaptable and groups learn to respond to threats specific to their environment. The ability to adapt to variable circumstances through social learning reflects the cognitive complexity characteristic of primates.

**ACKNOWLEDGEMENTS**

We would like to thank Z. Gezon for his help with statistical analysis, R. Chavez-Ulloa and R. Caslbeek for their insightful feedback, and the
members of Bio FSP 2013 who gave us valuable assistance in editing.

AUTHOR CONTRIBUTION
All authors contributed equally.

LITERATURE CITED


DIFFERENTIAL EVASIVE RESPONSE TO PREDATOR CALLS IN AUDITIVE MOTHs

TYLER E. BILLIPP, SAMANTHA C. DOWDELL, MARIA ISABEL REGINA D. FRANCISCO, ELISABETH R. SEYFERTH

Faculty Editor: Ryan Calsbeek

Abstract: Selective pressures exerted by predators favor evasive or defensive behaviors in prey. Many families of moths, for example, can detect the ultrasonic frequencies produced by echolocating bats. Selection calibrates moths’ hearing sensitivities to the frequency ranges of their primary predators. Therefore, the evasive behavior of moths with ears (auditive moths) should change with the potential predation threat posed by a given bat call. We hypothesized that auditive moths would exhibit the greatest evasive response to insectivorous bat calls, a decreased response to omnivorous bat calls, and the least response to non-insectivorous bat calls. We recorded evasive behavior of auditive moths when exposed to one of three bat calls compared to a control treatment of no sound. We found that the relative amounts of time spent performing evasive behaviors varied significantly with treatment and that the number of changes in flight pattern per treatment decreased as wingspan increased. Our results revealed a general difference in evasive response between treatments and suggest that maneuverability is limited by wingspan. Large moth species may compensate for their limited maneuverability by being more sensitive to ultrasonic frequencies compared to smaller moths, demonstrating how selective pressures for predator avoidance may vary with morphology.

Key words: echolocation, moth auditory characteristics, predator evasion, predation pressure

INTRODUCTION

Selective pressures exerted by predators favor the development of more effective evasive or defensive abilities in prey. However, anti-predator behaviors decrease time and energy available for other fitness-enhancing activities such as foraging and reproduction (Lima 1998; Fullard and Yack 1993). Prey that respond selectively to potential predation threats may minimize the costs of unnecessary evasion (Buss 2005).

Many families of moths are capable of detecting the ultrasonic frequencies produced by echolocating bats with simple “ears” called tympanal organs (Fullard 1988; Miller and Surlykke 2001). Hearing organs evolved in some moths prior to the evolution of bats, but at least three moth families independently evolved tympanal organs in direct response to the selective pressure of predation by echolocating bats (Fullard 1994; Kristensen 2012). Moths that can hear (hereafter “auditive moths”) may demonstrate evasive behaviors such as dives, turns, loops, and abrupt changes in direction when exposed to sources of ultrasound (Roeder 1966; Bennett 1971). Auditive moths are most sensitive to the range of ultrasound used by their primary predators, lepidoptivorous bats (Fullard and Belwood 1988; Fullard 1988) which likely reflects the strong selective pressure to differentiate calls of moth-eating bats from calls of all other bats. Therefore, moths may exhibit differential behavioral response to varying levels of predation threat.

We hypothesized that the evasive behavior of auditive moths would increase with the relative potential predation threat presented by a given bat call. We predicted that auditive moths would exhibit the most evasive behaviors when exposed to exclusively insectivorous bat calls, fewer evasive behaviors when exposed to omnivorous bat calls, and fewest evasive behaviors when exposed to non-insectivorous bat calls. Differential response should maximize the cost-benefit relationship between responding to actual threats and gaining adequate reproductive and foraging opportunities.

METHODS

We conducted our experiment on January 16, 2013 at the Palo Verde Biological Station in Guanacaste, Costa Rica. We captured six moths on a white sheet illuminated by a UV light and held them individually outside a laboratory with access to sugar and water. Trials took place in an unlit...
laboratory after dusk in order to avoid exposure to outside light sources.

For the experimental treatments, we downloaded the echolocation calls of three bat species commonly found in Palo Verde: the Davy’s Naked-Backed Bat (a native lepidoptivorous bat), the Fringe-lipped Bat (a native omnivorous bat), and the Common Vampire Bat (a native sanguivorous bat) from an online database (The Cornell Lab of Ornithology 2012). We assigned letters to each treatment (A=omnivorous bat, B=vampire bat, C=lepidoptivorous bat, D=no bat call) such that observers would not be biased by knowledge of treatment during observations. For each treatment, we haphazardly selected one moth, released it, and poked it lightly to stimulate phototaxis toward one white LED headlamp hung in the center of the ceiling. We played the bat call and began data collection when the moth reached a distance of one meter away from the headlamp. We played bat calls at full volume on repeat for one minute on a 13” MacBook Pro laptop with the screen darkened, and observed moths with red LED headlamps.

To measure behavioral response, we recorded time spent flying straight, time spent spiraling (flying in a series of circles), time spent not flying (landed on any surfaces), number of drops (falling straight downward with little wing movement), and direction of flight (towards or away from the bat call source). We classified negative phonotaxis, spiraling, and dropping as post hoc categories of evasive behaviors following Roeder (1966) and Bennett (1971). We exposed each moth to all four treatments in random order with three minutes of “recovery” (with the headlamp off and no bat call playing) between treatments. We based this recovery time on our own observations of how long the moth took to stop evasive behavior following preliminary trials. After the four treatments, we collected each moth, killed it with ethanol fumes, and identified it to family, confirming the presence of tympana using the DELTA interactive key (Watson and Dallwitz 2000). We measured wingspan and calculated the number of changes in flight pattern.

To investigate whether the major components of moth flight pattern varied by bat call type, we performed an ANOVA for time spent engaged in each individual behavior (spiraling, flying straight, or not flying) by bat call treatment. We used a chi-square to measure the effect of treatment on the relative frequencies of spiraling, flying straight, and not flying. We also used regression to estimate the relationship between wingspan and numbers of changes in flight pattern.

RESULTS
We found that the relative amounts of time spent spiraling, flying straight, and not flying varied significantly among treatments (Fig. 1; $\chi^2=105.27$, df=6, $P<0.001$). We also found that the number of changes in flight pattern decreased significantly as moth wingspan increased, regardless of species (Fig. 2; $r^2=0.19$, $P=0.03$). We did not find any significant differences in time spent landed, flying straight, or spiraling as a function of treatment ($F_{3,20}=1.68$, $P=0.20$; $F_{3,20}=1.33$, $P=0.29$; $F_{3,20}=0.92$, $P=0.45$, respectively).
DISCUSSION
We found that relative proportions of time spent on each flight behavior differed when moths were exposed to bat calls that represented varying levels of predation threat (Fig. 1). Although we did not find significant differences in separate flight behaviors by bat call, our results suggest that moths change their overall flight pattern in response to treatment. Due to the nature of the non-parametric analysis, we were unable to distinguish the nature of relationships between bat call types and specific behaviors. Future studies could further elucidate the response of auditory moths to bat calls by comparing evasive behavior in moths with hearing organs covered and uncovered.

The negative relationship between body size and number of changes in flight pattern (Fig. 2) suggests that small size either facilitates or is partially driven by selection for increased maneuverability in flight (Casey 1981). Maneuverability might enable complicated and unpredictable flight patterns that could be a major advantage in evading attacking bats (Roeder 1974). Large moth species may compensate for their limited maneuverability by being more sensitive to ultrasonic frequencies than are smaller moths (Surlykke et al. 1999). Further research should investigate evasive behaviors in response to predatory calls across a wider moth size range in a single species of auditory moth.

We found that moths differed in their responses to different bat calls. Though we cannot assess the biological importance of differences in response among treatments, the ability to tailor evasion response to threat level could lead to selective advantage in bat encounters. This ability would also minimize the loss of time and energy incurred by unnecessary evasion (Lima 1998). Additionally, smaller wingspan may have contributed to increased maneuverability, another advantage against bat predation. Large moths are limited in maneuverability by morphology, but they may compensate by being more sensitive to ultrasonic frequencies than smaller moths. Pressure to detect predators audibly in order to increase reaction time may then increase as wingspan increases and maneuverability decreases. Maneuverability in small moths and increased auditory sensitivities in large moths would therefore serve as an example for how selective pressure for predator avoidance can vary with morphology.

ACKNOWLEDGEMENTS
We would like to thank Zachariah Gezon, Ramsa Chaves-Ulloa, and Ryan Calsbeek for their guidance and assistance throughout the brainstorming and methodology development processes. We would also like to thank Sergio Alberto Padilla Álvarez for sharing his expertise regarding native bats.

AUTHOR CONTRIBUTIONS
All authors contributed equally to experimental design, execution of experiment, and writing of manuscript.

LITERATURE CITED


ONE ROBBER, TWO VICTIMS: EXPLOITATION OF *Apis mellifera* AND *Eichhornia crassipes* PLANT-POLLINATION MUTUALISM THROUGH NECTAR ROBBING

GILLIAM A. O. BRITTON, COLLEEN P. COWDERY, JIMENA DIAZ, EMILIA H. HULL, ELIZA W. HUNTINGTON, ELLEN T. IRWIN

Faculty Editor: Ryan Calsbeek

**Abstract**: Mutualisms are prevalent in nature, but are often susceptible to exploitations from a third party. Stingless bees (*Meliponinae*) exploit the plant-pollinator mutualism between Africanized honeybees (*Apis mellifera*) and water hyacinth (*Eichhornia crassipes*). We explored this relationship by experimentally excluding stingless bees from water hyacinth inflorescences and comparing pollen receipt and honeybee visitation of unmanipulated, nectar-robbed flowers to that of experimentally unrobbed flowers. We examined the effect of this exploitation on both honeybee foraging and water hyacinth pollination and found that the number of honeybee visitations and quantity of pollen receipt decreased in the presence of nectar robbers. Our results also showed that inflorescences protected from both stingless and honeybees had the highest pollen count, suggesting that self-pollination occurs in the absence of pollinators. Nectar robbing by stingless bees has a negative effect on both honeybees and water hyacinth as it increases the cost of foraging for honeybees and decreases cross-pollination in water hyacinths. Our results shed light on the three-way relationships among these species, and provide further understanding of multi-species interactions.

**Key words**: *Apis mellifera*, *Eichhornia crassipes*, *Meliponinae*, multi-species interactions, nectar robbing

**INTRODUCTION**

Mutualisms are relationships in which participants benefit from interactions with one another; each organism’s investment produces benefits that outweigh the cost of investment (Connor 1995). Mutualisms are prevalent in nature, but are susceptible to exploitations from a cheating third party that steals resources from mutualists (Bronstein 2001). Many studies have identified exploited mutualisms and highlighted nectar robbing in plant-pollinator relationships as a model system (Bronstein 1991, Roubik 1985). Nectar robbing describes the act of a potential pollinator chewing through to the nectar reservoir at the base of a flower and consuming nectar while avoiding pollinating the flower (Irwin and Brody 1999), stealing resources from both the plant and its pollinators.

Despite extensive study on nectar robbing, there remains limited knowledge of its effects on the mutualism between Africanized honeybees (*Apis mellifera*) and water hyacinth (*Eichhornia crassipes*). Africanized honeybees pollinate the water hyacinth by entering the flower at the opening to reach the nectar reserve, brushing the anthers *en route*, and depositing pollen on the stigma (Gurevitch et al. 2002). In contrast to honey bees, stingless bees avoid contact with the stigma by reaching the nectar reserves at the base of the flower (Irwin and Brody 1999). In this study, we observed stingless bees (*Apidae*, *Meliponinae*) nectar robbing water hyacinth flowers prior to honeybee visitations, thereby exploiting the plant-pollinator mutualism between Africanized honeybees and water hyacinth. We hypothesized that nectar robbing by stingless bees would decrease honeybee visitation and thus reduce the amount of pollen received by the water hyacinths. Exploitation of the mutualism between water hyacinth and honeybees by stingless bees should therefore reduce the net benefit received by both members of the mutualism.

**METHODS**

We conducted this study in January, 2013, in Palo Verde National Park, Costa Rica. To test the effects of nectar robbing on water hyacinth visitation by honeybees, we experimentally manipulated both stingless bee and honeybee access to water hyacinth flower clusters (inflorescences). We assigned four treatments to the water hyacinth inflorescences: Un-robbed (U), Robbed (R), No bees included (N) and a control treatment that allowed normal bee visitations (C). For treatment U, unopened inflorescences were bagged before dawn the day before the study to
Prevent nectar robbing, and bags were removed at 7:30 am on the day of study to allow for honeybee visitation. For treatment R, unopened inflorescences were bagged on the day of study when the flowers first opened, or when the first honeybee visited the flower (roughly 7:30 am), allowing stingless bees to rob nectar before the pollination period but preventing honeybee visitation. Treatment C inflorescences were left unbagged during the experiment to allow both nectar robbing and pollinator visitation. Treatment N inflorescences were bagged at the same time as treatment U and left bagged throughout the experiment to exclude all visitors and prevent both nectar robbing by stingless bees and honeybee visitation.

Before sunrise on January 15th, we haphazardly selected 24 water hyacinth inflorescences near the shore of the Palo Verde marsh (sample sizes: U=5, R=5, C=8, N=6). On January 16th, we haphazardly chose 19 inflorescences (sample sizes: U=5, R=5, C=5, N=4). There was incidental variation in sample size between the two collection days to end up with a total of 10 inflorescences for treatments U, R, and N. The control treatment was largest (N=13) to be certain of baseline visitation rate and pollen receipt level in an unmanipulated scenario. Treatments were randomly assigned to inflorescences by drawing numbers from a bag. During honeybee foraging time (7:30-8:45 am), each of X observers counted the number of honeybee visits and the number of flowers visited per honeybee to a particular inflorescence for treatments C and U (the unbagged treatments that remained exposed for honeybee visitation). Nectar robbing in treatment R was not directly observed as it occurred in early morning low light, but flowers in the R treatment were examined and confirmed to have nectar-robbing holes at their bases at the end of the experiment. After the observation period, we counted the number of flowers per inflorescence, and approximated flower density within a 1 m radius around the inflorescence. Stigmas from the top three flowers of each observed inflorescence were collected and used to prepare pollen slides using basic fuchsin jelly (Kearns and Inouye 1993). Pollen grains were counted using a compound microscope at 40X.

Statistical Analysis

We confirmed that our data were normally distributed by examining the normal quantile plot of each distribution. We identified an inflorescence from the control treatment that bloomed 30 minutes into the sampling period as a statistical outlier. From observation, the opening of a new flower at a time when others already had depleted nectar resources may have resulted in an unusually high visitation rate. In the results that follow, we present all analyses both including and excluding this outlier. We used ANCOVA to test how the average number of flowers visited per bee varied by treatment, including the number of flowers per inflorescence as a covariate. We confirmed equality of variances among treatments before we ran a two-sample t-test using the average number of flowers visited per bee as the dependent variable and experimental treatment as the single factor. We combined data from both days, after testing to ensure that the day of data collection was not a significant covariate. To analyze the effect of our four treatments on pollen count we used a one-way ANOVA with Tukey HSD post hoc comparisons. Finally, we used regression to test whether pollen grains per stigma could be predicted by average number of flowers visited per bee. We used JMP 10.0 statistical software for all analyses.

RESULTS

Preliminary analyses revealed that the number of flowers per inflorescence was not a significant covariate ($F_{2,17} = 6.26, P = 0.460$) and is thus not considered further. We found that the average number of flowers visited per bee was significantly higher in unrobbed flowers than flowers that were exposed to bees all morning ($t_{22}=2.22, P=0.020$; Table 1; Figure 1).

Table 1. Mean flower visits per bee was higher in the unrobbed treatment than the control; the difference was greater after excluding an outlier.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean (visits)</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrobbed</td>
<td>2.09</td>
<td>0.341</td>
</tr>
<tr>
<td>Control</td>
<td>1.24</td>
<td>0.298</td>
</tr>
<tr>
<td>Control, w/o outlier</td>
<td>0.965</td>
<td>0.135</td>
</tr>
</tbody>
</table>
The difference was more pronounced when we removed the outlier from the control treatment ($t_{16}=3.50, P=0.003$; Table 1; Figure 1).

![Graph showing average number of flowers visited per bee for control and unrobbed treatments.](image)

Figure 1. The average number of flowers visited per bee is higher for inflorescences that were bagged until the flowers opened (unrobbed treatment), than for the control, which was unbaggged all day. The box marks an outlier we tested both with and without. Data collected Jan 15 and 16, 2013 in Palo Verde National Park, Guanacaste, Costa Rica.

We found that pollen count increased linearly with flowers visited per bee ($r^2=0.20, P=0.05$; Figure 2). The relationship was likewise stronger after the outlier was removed ($r^2=0.40, P=0.005$). The mean pollen count differed by treatment ($F_{3,42}= 5.05, P = 0.05$; Table 2): the flowers that were bagged all day had a significantly higher pollen count than both control ($P=0.012$) and robbed flowers ($P=0.008$; Figure 3). We also found that the unrobbed treatment had a higher mean pollen count than control and robbed flowers, but this difference was not significant ($P=0.28$, $P= 0.40$, respectively).

![Graph showing pollen count increases linearly with average number of flowers visited per bee.](image)

Figure 2. Pollen count increases linearly with the average number of flower visits per bee to an inflorescence, regardless of treatment. The box marks an outlier we tested with and without.

![Graph showing mean pollen count per flower by treatment.](image)

Figure 3. The mean pollen grains per stigma of flowers in the ‘no pollinator’ treatment is significantly higher than the mean pollen count of both ‘control’ and ‘robbed’ treatments (Tukey’s HSD $p=0.012$, $p=0.008$, respectively). Bars show standard error and different letters above the bars indicate significant difference.

Table 2. The mean pollen count per flower differed by treatment—the no pollinator treatment had significantly more pollen than both control and robbed treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean (grains)</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.7</td>
<td>5.1</td>
</tr>
<tr>
<td>No pollinators</td>
<td>45.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Robbed</td>
<td>16.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Unrobbed</td>
<td>32</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Dartmouth Studies in Tropical Ecology 2013
DISCUSSION
Our results demonstrate that nectar robbing by stingless bees has a negative effect on both honeybees and water hyacinths. Stingless bees remove nectar from the water hyacinth and deplete nectar reserves for honeybees without pollinating the plants. We demonstrated that both the average number of flowers visited per bee to a hyacinth inflorescence and the pollen count per stigma was lower in robbed than unrobbed flowers, supporting our hypothesis that nectar robbing by stingless bees (*Meliponinae*) negatively affects both *A. mellifera* and *E. crassipes*. Our results imply that nectar robbing increases the cost of foraging for honeybees. We speculate that lower numbers of repeated visits indicate that honeybees gain less nectar from robbed plants and must therefore visit more inflorescences to find sufficient nectar. In addition, there likely was reduced heterospecific cross pollination, which may be an indicator of lower reproductive success in the presence of stingless bees (Schemske and Pautler 1984).

We also found that the mean pollen count was higher for plants that were excluded from all bees compared to plants that were exposed to both honey- and stingless-bees or to those that were only exposed to robbers. This was opposite the pattern that we anticipated given the lack of bee visitations. There was no significant difference in the mean pollen count between unrobbed flowers and flowers in the bee exclusion treatment. The greater amount of pollen found in the bee exclusion treatment could be explained by the phenomenon in which autonomous self-pollinating plants, like the water hyacinth (Mulcahy 1975), can delay selfing until the end of anthesis and thereby avoid unnecessary self-pollination while ensuring seed production when pollinators are scarce (Klips and Snow 1997). Addition of pollen to a stigma during anthesis causes the stigma to become erect, moving it further away from the anthers and lowering the chances of self-pollination once it is cross-pollinated (Klips and Snow 1997). Therefore, experimental treatment N may have had a high pollen count due to the lack of pollinator presence eliciting the post-anthesis self-pollination process.

It is important to note that the honeybees in this study are invasive Africanized honeybees, whereas the stingless bees are native to Costa Rica. Africanized honeybees entered Costa Rica in 1983 (Frankie et al. 1997), and are thought to be reducing the population of stingless bees throughout Central and South America because of their superior competitive abilities, such as their relatively larger body size (Roubik 1980). In our study area, however, previous studies have suggested that there is little evidence that Africanized bees are reducing the numbers of native bee species (Frankie et al. 1997). Nevertheless, Africanized bees may be spatially and/or temporally displacing some bee species (Frankie et al. 1997). This is supported by our observations in the field: stingless bees were much more active before and after honeybees had finished the majority of their foraging, many stingless bees were pushed out of flowers by larger honeybees, and stingless bees pollinated flowers in the absence of honeybees. A final consideration of the effect of invasive honeybees is that it forces the native stingless bee to nectar rob in order to compete with a dominant pollinator. Nectar robbing may not be the most advantageous strategy for stingless bees, as water hyacinths have single-day flowers (Barrett 1977). Nectar robbing is less profitable if flowers only last a single day, because after opening a hole to the nectar source, robbers need no additional investment to continue robbing flowers that produce nectar for longer periods of time (Roubik 1982). Future studies could test the effect of Africanized honeybees on the foraging behavior of stingless bees. This would help decipher whether nectar robbing is the preferred behavior of stingless bees or whether they have adopted it as a method to compete with the larger invasive honeybees.

Finally, water hyacinth is an invasive species, native to lowland South America. (Barrett et al. 2008). Considering that there are no native honeybees in the area (Frankie et al. 1997), it would be interesting to investigate how the introduced honeybees may be facilitating the proliferation of the water hyacinth. Our findings open the door to future studies that could further our understanding of the interaction between multiple invasive species and highlight the complexity of the three way relationship among Africanized honeybees, stingless bees, and water hyacinth.
ACKNOWLEDGEMENTS
We would like to thank the staff of Palo Verde National Park for providing sustenance, and R. Calsbeek, Z. Gezon and R. Chaves-Ulloa for their assistance in experimental design, general guidance and support.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
Abstract: Resource and energy limitations force tradeoffs between reproduction and other aspects of fitness. To investigate these tradeoffs we tested the effects of abiotic factors and relative investment in aboveground and belowground growth on reproductive status of the fern Thelypteris. We predicted that the aboveground-to-belowground (A/B) biomass ratio would decrease with increased wind exposure due to the greater need for investment in the root system for stability. We also hypothesized that fertility would increase with A/B ratio because increased investment in a belowground root system would detract from aboveground growth and would result in lower spore production. Contrary to expectation, we found no relationship between wind exposure and A/B biomass ratio. We also found that Thelypteris fertility decreased with the ratio of A/B biomass. Because A/B was a more important factor for fertility than either aboveground mass or belowground mass alone, we speculate that a tradeoff in energy investment was a driving factor behind fertility. Fern fertility also increased with average wind speed. This could suggest that exposure to the humid winds of the cloud forest allows greater investment in spores. Environmental conditions around ferns may affect nutrient and energy limitations in individuals and force tradeoffs between investment in survival and reproduction that influence individual fitness.

Keywords: fertility, microclimate effects, Thelypteris, tradeoff theory

INTRODUCTION
Reproduction is energetically demanding for organisms but is an essential part of individual fitness. Resource limitation combined with the high cost of reproduction means that organisms must tradeoff energy allocation between reproduction and survival when trying to maximize fitness. Tradeoffs must occur whenever the energy available is less than the individual’s total energetic requirements in a given period of time (Williams 1966). For example, fecundity in female guppies is correlated with size, but investment in body growth reduces energy available for reproduction and forces a tradeoff (Reznick 1983). Likewise, there is a negative relationship between reproductive capacity and flight capacity in adult male sand crickets (Nespolo et al. 2008).

Here, we investigate how tradeoffs between reproduction and growth are affected by environmental factors. We studied Thelypteris (Polypodiales: Thelypteridaceae), a genus of widespread perennial fern (Moran 2002). Species within this genus can exhibit morphological variation due to differences in microhabitat (Hill 1971, Bartsch and Lawrence 1997) making them ideal for research into the effects of abiotic factors on growth and fertility over small spatial scales.

We hypothesized that greater exposure to wind would result in increased investment in root production for stability, lowering investment in aboveground growth. Thus, the ratio of aboveground-to-belowground (A/B) biomass should decrease with increasing wind exposure. We also hypothesized that increased investment in roots would leave less energy and resources available for reproduction, meaning that fewer spores would be produced. Thus, we predicted that fertility would increase with A/B biomass ratio.

METHODS
Data Collection
We haphazardly selected 17 ferns from the genus Thelypteris on the 22-23rd of January, 2013 in the forests surrounding the Monteverde Biological Station, Puntarenas, Costa Rica. To control for potential effects of altitude on investment, all ferns were collected from an elevational range of 1783-1815 m. We selected ferns of similar size and recorded elevation and latitude/longitude using Garmin GPSmap 76CSx. We measured wind speed at each sampling location on January 23rd between 8:50-10:00am and again between 1:25-3:00pm, by holding a Kestrel 3000 anemometer at the height of the tallest frond and recording average and highest wind speeds over four
minutes. We measured soil pH (using a Rapitest 4-Way Analyzer), soil moisture (using a Tenax Moisture Meter), and soil slope (using a Suunto clinometer) directly adjacent to each fern. We took a picture of the canopy from the position of the plant and used ImageJ (Wayne Rasband 1.45s) software to calculate percentage canopy cover. We uprooted all plants, bagged them, and brought them to the laboratory. We calculated percentage of leaflets that were fertile (hereafter referred to as “fertility”) by tallying all non-spore and spore-bearing leaflets greater than 1 cm in length from each fern. We then separated all fronds from their roots, placed all plant parts in a drying oven (60°C) for 8 hours, and used an aeADAM Highland HCB123 scale to measure A/B dry biomass.

Statistical Analyses
We excluded four ferns from analysis because two were not Thelypteris ferns upon closer inspection and two had root growth that was interrupted by a plastic sheet and human foot traffic. To determine whether A/B dry biomass ratio was related to any of our independent variables, we performed a stepwise regression of fertility against all parameters (elevation, average wind speed, maximum wind speed, soil pH, soil moisture, soil slope, percent canopy cover) comparing all possible model subsets using the Akaike Information Criterion (AIC) values to determine the most parsimonious model (Cavanaugh 2007). To analyze factors influencing fertility, we performed a stepwise regression of fertility against all parameters (elevation, average wind speed, maximum wind speed, soil pH, soil moisture, soil slope, percent canopy cover, A/B dry biomass) using AIC values to determine the most parsimonious model. All data fulfilled the assumptions of the statistical tests used. We used JMP 10.0 (SAS Institute, Inc. 2012) for all statistical tests.

RESULTS
We found no relationship between A/B biomass ratio and any of our independent parameters. However, A/B dry biomass ratio and average wind speed both explained a significant portion of the variation in percentage of leaflets that were fertile in a multiple regressions analysis ($r^2=0.676$, df=9, $P=0.0063$). Percent fertility decreased with increasing A/B biomass ratio (Fig. 1, Table 1) while percent fertility increased with average site wind speed (Fig. 2, Table 1).

![Figure 1. The percentage of leaflets carrying spores decreased with the ratio of aboveground to belowground dry biomass after controlling for variation in average wind speed.](image-url)
Monteverde

Table 1. A/B biomass ratio and average wind speed exposure significantly explained variation in fertility of *Thelypteris*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/B Biomass</td>
<td>-0.247</td>
<td>0.014</td>
</tr>
<tr>
<td>Average Wind Speed</td>
<td>0.295</td>
<td>0.046</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Contrary to our hypothesis, we found no relationship between wind exposure and A/B biomass ratio in *Thelypteris*. Also, while we had predicted that increasing mass of fronds relative to root mass would result in increased fertility, we actually found that the increasing A/B mass led to decreasing fertility. Because the ratio of A/B dry biomass explained more variation in fertility than did aboveground mass or belowground mass alone, we hypothesize that a tradeoff in energy investment was a driving factor behind the fertility of *Thelypteris*. The tradeoff in the relationship between A/B and fertility could be explained by the cyclical pattern of spore production found in these ferns (Bartsch and Lawrence 1997): some ferns invest in greater frond growth one year to build up energy that is stored in the roots; the following year, frond growth is reduced and the stored energy is used for spore production (Emery et al. 1994). This pattern of growth and reproduction suggests that ferns must selectively allocate energy and resources between the costly activities of leaf production and spore production. In addition, the ability to store energy in root systems might explain why a relative increase in the portion of biomass due to roots would allow greater fertility.

We found that fertility increased with wind exposure and that wind exposure varied across small spatial scales. The ferns in our study had developed and produced fertile leaflets during the montane forest’s misty-windy season that takes place from November through January; during this season, winds are the primary way in which moisture is carried into the ecosystem (Nadkarni et al. 1995). Tropical ferns in the closely-related genus *Polypodium* are capable of absorbing water through their leaves and their roots (Stone 1957). If this ability is shared by *Thelypteris*, humid winds might explain the increased fertility of wind-exposed plants despite the lack of a relationship between soil moisture and fertility in our results.

Our findings provide support for a trade-off between investment in reproduction and growth in...
Thelypteris. The balance between reproduction and survival is especially important for Thelypteris and other organisms that reproduce multiple times in their lifetime, since enough energy and resources must be reserved for survival in order to preserve life during and after reproduction (Williams 1966). Interestingly, we also found that variance in abiotic factors to which an individual is exposed affects reproductive ability across an extremely small geographical scale. Our results suggest that even relatively minor changes in environmental conditions may affect the nutrient and energy limitations in individuals that lead to life history tradeoffs.

ACKNOWLEDGEMENTS
We would like to thank the staff of Monteverde Biological Station for their support and for the use of their oven for drying samples and CIEE for providing soil probes and an anemometer. We would also like to thank Zachariah Gezon, Ramsa Chaves-Ulloa, and Ryan Calsbeek for their guidance and assistance.

AUTHOR CONTRIBUTIONS
All authors contributed equally to experimental design, execution of experiment, and writing of manuscript.

LITERATURE CITED
THE EFFECT OF HUMMINGBIRD SIZE ON TERRITORIALITY AND FORAGING STRATEGY

COLLEEN P. COWDERY, EMILIA H. HULL, ELLEN T. IRWIN, MOLLY P. PUGH, MARIA ISABEL FRANCISCO

Faculty Editor: Ryan Calsbeek

Abstract: Foraging is a costly activity, so organisms should optimize the amount of energy spent in relation to the value of food. Optimal foraging theory predicts that territorial animals should forage a little at one time so that they can continue to exploit their resource in the long-term. Non-territorial animals should maximize immediate gains by consuming as much of the resource as possible. Morphological characteristics such as body size often affect which foraging strategies organisms employ. In order to examine how size affects foraging strategies, we compared the aggressive tendencies and visitation rates for four species of hummingbird, two large and two small. We found that large hummingbirds foraged fewer times per visit and were more likely to be aggressive toward other species, while small hummingbirds were more likely to be aggressive toward their own species. Our results suggest that large hummingbirds are more likely to defend a single resource against other species, while small hummingbirds forage opportunistically and try to consume as much nectar as possible in the face of intraspecific competition. Our results demonstrate that optimal foraging strategies are at least partly driven by morphological characteristics.

Key words: Callistemon viminalis, nectar availability, optimal foraging theory, Salvia pteroura, Tachypheta jamaicensis, Trochilidae

INTRODUCTION

Foraging is a costly activity, so organisms should minimize costs so that energy spent is optimized in relation to the energy value of the food (Hainsworth and Wolf 1972). Organisms have evolved different strategies, such as territoriality, to maximize their energy intake while minimizing their foraging costs. Territorial animals defend a territory against others to gain almost exclusive access to its resources (Hixon et al. 1983). Optimal foraging theory predicts that territorial animals should forage at a lower short-term efficiency to acquire a higher long-term yield. Non-territorial animals, however, do not look beyond immediate short-term gains and therefore consume as much of the resource as possible (Pyke et al. 1977).

Morphological characteristics such as body size often affect which foraging strategies organisms employ (Weinbeer and Kalko 2004, Hainsworth and Wolf 1972). We examined the relationship between body size and foraging strategy in hummingbirds, which have a high metabolism and must therefore forage efficiently (Best and Bierzychudek 1981). Territorial hummingbird species spend much of their time engaging in defensive behaviors such as perching and chasing; foraging is only a small part of their daily energy budget (Feinsinger and Chaplin 1975). Non-territorial hummingbirds spend more time foraging, which is energetically demanding (Hainsworth 1981). Previous studies have found that smaller hummingbirds have greater foraging efficiency than large ones (Hainsworth and Wolf 1972). However, large hummingbirds’ success in aggressive interactions may compensate for their low efficiency by allowing them to exclude smaller hummingbirds from food sources (Hainsworth and Wolf 1972).

We hypothesized that larger hummingbirds would be more territorial than small hummingbirds. If larger hummingbirds are more territorial, they should forage to maximize their long-term yield, while smaller hummingbirds should employ a more short-term strategy. We hypothesized that larger hummingbirds would forage fewer times in one visit to a plant than smaller hummingbirds would. The optimization of foraging based on body size could shed light on the relationship between morphological characteristics and energy budgeting.

METHODS

We collected our data on January 22-24, 2013, in Monteverde, Costa Rica. We selected three species...
of plant - weeping bottlebrush (*Callistemon viminalis*), blue porterweed (*Tachytarpheta jamaicensis*) and deep purple sage (*Salvia pteroura*) - where we had previously observed hummingbird activity. We measured the number of flowers for five haphazardly chosen inflorescences, as well as the number of inflorescences within a 0.5 m radius from the center of the focal plant. As each plant had relatively high inflorescence density, we determined the area measured was sufficient to estimate resource density. We observed each plant for 20 minute trials in the morning (06:00-07:00, 07:30-09:30) on Jan 23 and 24, and in the afternoon (14:30-17:30), on Jan 22 and 23, with 10-minute breaks between trials. We recorded the number of individual visits to the plant per hummingbird species (“visits”), how many inflorescences at a plant were visited per individual hummingbird (“repeated visits”), and aggressive interactions displayed during each trial, noting the species of the participants. We classified these interactions into two categories: aggressive (when the observed hummingbird chased away another hummingbird) and retreating (where the observed hummingbird was chased away). If a visit continued past the end of the 20-minute trial, we continued recording data for that bird until the visit ended.

Four species of hummingbirds visited our plants: coppery-headed emerald (*Elvira cupreiceps*), striped-tailed (*Eupherusa eximia*), magenta-throated woodstar (*Calliphlox bryantae*) and green violet-ear (*Colibri thalassinus*). Small birds (coppery-headed emerald and magenta-throated woodstar) were 7.5-9.0 cm from tip of beak to tip of tail and weighed 3.0-3.5 g; large birds (striped-tailed and green violet-ear) were 9.5-10.5 cm and weighed 4.0-5.0 g (Stiles and Skutch 1989).

We conducted a total of 15 trials for each plant in a randomized order. To measure nectar availability, we bagged three inflorescences per plant at 05:45 on Jan 22 using Ziploc® bags to exclude all visits from hummingbirds throughout the morning observation period. At 09:30, using a 50 µL capillary tube and a caliper, we measured the quantity of nectar for five flowers on each of the three inflorescences at each plant. In addition, using a Sugar/Brix Refractometer with ATC, we measured the percent sugar of each nectar sample.

We repeated this method for the afternoon observation period, bagging three different inflorescences at 14:15 and measuring nectar and percent sugar at 17:30. We then calculated the calories per m² for each plant by multiplying the average nectar volume, percent sugar and flower density per area.

We were unable to measure the nectar content of porterweed, possibly because of the low volume or high viscosity of the nectar. We found literature values for porterweed nectar volume and sugar content (Demcheck 2003), but the source did not describe the manner in which data were collected. Thus, we decided not to calculate calories per unit area or perform any statistical analyses beyond reporting the values (Table 1). All three nectar qualities fall within a typical range for pollinator nectar sugar content (Baker 1975).

**Statistical Analysis**

To determine how the mean number of repeated visits to plants varied by hummingbird body size, we performed a Wilcoxon test. To test how the mean number of total visits varied by hummingbird size and plant species, we performed another Wilcoxon test. Nonparametric tests were used because data did not fit the assumptions of parametric tests. We used JMP 10.0 for all statistical analyses.
RESULTS

The average number of repeated visits varied significantly with hummingbird size for all plant species ($\chi^2 = 12.98$, df = 1, $P = 0.0003$; Figure 1). Large hummingbirds had more interspecific aggressive interactions ($\mu = 0.27 \pm 0.06$ (SE); Figure 2) than small birds ($0.02 \pm 0.05$ interactions); small birds had more intraspecific aggressive interactions ($0.07 \pm 0.02$ interactions), and we did not observe large birds exhibiting intraspecific aggression ($0.00 \pm 0.00$ interactions). Large birds retreated in few interspecific encounters ($0.01 \pm 0.04$ interactions) while small birds often retreated in interspecific encounters ($0.13 \pm 0.03$ interactions).

Large hummingbird visitation to the three plants varied significantly ($\chi^2 = 15.88$, $P = 0.0004$, df = 2, Figure 3). Large birds preferred bottlebrush first, porterweed second, and sage third. Small birds did not show significant plant preferences ($\chi^2 = 3.06$, $P = 0.22$, df = 2; Figure 3). Flower arrangement and nectar quality varied significantly as a function of plant species (Table 1). The difference in calories per m$^2$ between bottlebrush and sage was statistically significant ($\chi^2 = 11.19$, $P = 0.0008$, df = 1). Bottlebrush had lower sugar content per flower, but had more calories per m$^2$, while sage had higher sugar content per flower but fewer calories per m$^2$ (Table 1).

DISCUSSION

Our results supported our hypothesis that large hummingbirds employed territoriality as a foraging strategy. We found that large hummingbirds (striped-tailed and violet-eared) visited significantly fewer flowers per individual foraging visit to a plant (Figure 1). We also observed that large hummingbirds tended to divide their time between foraging for short intervals and scanning or defending their territories. Our data and observations suggested that large hummingbirds allocate more energy toward defense at the expense of foraging. By foraging less frequently but defending a territory, they can obtain a higher long-term yield (Pyke et al. 1977).

The lack of conflict between large hummingbirds may be the result of energy
Table 1. Plant-flower arrangement and nectar quality in the three species of plants.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Inflorescences per m²</th>
<th>Flowers per inflorescence</th>
<th>Flowers per 0.5m radius</th>
<th>% glucose (µ ± SD)</th>
<th>Nectar volume (µL; µ ± SD)</th>
<th>Calories per m² (Kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottlebrush</td>
<td>15</td>
<td>40</td>
<td>600</td>
<td>4.4 ± 4.08</td>
<td>6.04 ± 4.47</td>
<td>27.49 ± 4.7</td>
</tr>
<tr>
<td>Porterweed</td>
<td>60</td>
<td>7</td>
<td>420</td>
<td>27.05 ± 0.78</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Purple sage</td>
<td>40</td>
<td>10</td>
<td>400</td>
<td>2.65 ± 0.91</td>
<td>15.50 ± 10.72</td>
<td>4.11 ± 4.7</td>
</tr>
</tbody>
</table>

*Literature value

budgeting; both intruder and territory holder would suffer too high a potential cost if they engaged in an aggressive encounter (Dearborn 1998). However, because larger hummingbirds dominate smaller birds (Hainsworth and Wolf 1972), aggressive interactions with smaller birds are less costly; explaining why larger birds in our study were likely to successfully drive off the smaller hummingbirds (Figure 2).

We also found that small hummingbirds were the aggressors in primarily intraspecific encounters (Figure 2). Without clear-cut territorial boundaries, there may be greater opportunities for conflict due to competition. Small birds initiated fewer interspecific encounters, possibly due to their smaller size and greater probability of retreating from a conflict (Figure 2).

Large hummingbird preferred the bottlebrush, the plant with the highest caloric reward per meter squared, and remained almost exclusively in those areas. As territoriality is an expensive behavior, large hummingbirds may choose to defend the plants with the highest reward. There was no significant preference for flower species in the small hummingbirds, which suggests that they do not exclusively defend one plant, but rather forage more generally among plants with a variety of nectar volumes and sugar contents (Figure 3). Future studies might compare nectar volume and sugar content between hummingbird-pollinated plants, and clarify the relationships between reward preference and territoriality.

Our results demonstrate that hummingbirds optimize their foraging strategies according to body size. This variation in foraging strategies across sizes may illustrate the constraints that morphology places on energy allocation. Organisms that efficiently budget their energy can allocate more of it toward other fitness-enhancing behaviors such as reproduction and predator avoidance (Hainsworth 1981). Thus, understanding the factors relevant to energy allocation sheds light on the different ways organisms can maximize their fitness.

ACKNOWLEDGEMENTS
We would like to thank the staff of the Monteverde Biological Station, and R. Calsbeek, Z. Gezon, and R. Chaves-Ulloa for their support and their guidance in experimental design.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED

**BENEFITS OF FLUSHING RED FOR A TROPICAL TREE (ALFAROA COSTARICENSES)**

AMELIA F. ANTRIM, TYLER E. BILLIPP, GILLIAN A. O. BRITTON, ELIZA W. HUNTINGTON, AND KALI M. PRUSS

Faculty Editor: Ryan Calsbeek

*Abstract*: Plants must outcompete co-occurring species, allocate resources to maximize growth, and avoid predation to improve fitness. Delayed greening of new leaves is thought to be an adaptation for maximizing fitness in many tropical plants. However, the literature remains divided on the adaptive significance of flushing red. The numerous competing hypotheses include: 1) red leaves are adapted for growth in low-light in the understory, 2) plants invest fewer resources in new growth red leaves to limit loss of resources to herbivores, 3) red leaves act as a defense mechanism against herbivore activity, and 4) red leaves offer anti-fungal defense. In this study, we used the tropical, shade-intolerant tree *Alfaroa costaricensis*, to examine the role of delayed greening as an anti-herbivore defense. We hypothesized that green leaflets would sustain greater damage by herbivores than red leaflets because previous findings suggest that red color serves as a deterrent to herbivory. We also predicted that red leaflets would be less tough and contain lower sugar levels than green leaflets, since structural development and energetic investment are delayed until leaflet maturation. We showed that red leaflets are significantly less tough than green leaflets and have a greater sugar concentration than green leaflets. Despite lower toughness and higher sugar content, red leaves sustained less herbivore damage, suggesting that red coloration provides defense against herbivory. The ability to defend new growth against herbivory may explain the success of *A. costaricensis* as a light gap colonizer. Species race for limited space in light gaps, thus adaptations to maximize growth and minimize loss to herbivory are vital to outcompeting other successional species.

*Keywords*: *Alfaroa costaricensis*, antiherbivore defense, delayed greening

**INTRODUCTION**

Organisms are constantly expending energy; growth, protection and reproduction are energetically costly but necessary to individual and species survival. Thus, to maximize survival, organisms must divide resources between energy expenditure and conservation. For example, plants must balance resource allocation between growth and defense to outcompete co-occurring species. Different species have unique ways of investing their resources to optimize survival.

Many tropical plant species flush their young leaves red, delaying greening and nutrient investment until the leaf is mature (Sestak 1985; Kursar and Coley 1992a). The red color in young leaves is caused by the presence of anthocyanins (Harborne 1967), which are secondary metabolites (Coley and Barone 1996). Anthocyanins are associated primarily with shade-tolerant plant species and are only present in immature leaves (Coley and Barone 1996).

The mechanism of delayed greening appears to provide an evolutionary advantage to plants since many disparate taxa have converged independently upon the same tactic (Kursar and Coley 1992b). However, the adaptive significance of this trait is debated, in particular concerning the importance of red flushing (the production of new leaves). Four main hypotheses have been proposed to date: 1) red leaves are adapted for growth in low-light in the understory (Kursar and Coley 1992c), 2) plants invest fewer resources in red...
leaves to limit loss of resources to herbivores (Kursar and Coley 1992b,c), 3) red leaves deter herbivore activity (Coley and Aide 1989, Karageorgou and Manetas 2006), and 4) red leaves offer antifungal defense (Coley and Aide 1989).

Across all plant species, 60 to 80 percent of lifetime predation occurs on immature leaves (Coley and Aide 1991; Coley and Barone 1996); young leaves usually have high nitrogen and water content and low toughness (Kursar and Coley 1992c), making them an ideal resource for herbivores. Therefore, if herbivory rates are lower on red leaves, which otherwise appear ideal for consumption, red coloration may serve as an anti-herbivore defense. Various studies claim that anthocyanins are toxic to herbivores (Karageorgou and Manetas 2006), while others suggest that the red coloration is less visually appealing to herbivorous insects (Juniper 1993). Thus, there is abundant reason to think that red leaves may sustain less herbivory compared to green leaves.

We tested the role of delayed greening as an anti-herbivore defense mechanism using Alfaroa costaricensis in the cloud forest of Monteverde, Costa Rica. We hypothesized that green leaflets would have more herbivore damage than red leaflets. We also predicted that red leaflets would be less tough than mature green leaflets as they have not reached full size. Finally, we expected red leaflets to contain lower sugar levels, as energetic investments are delayed until leaf maturity.

METHODS
We used leaves from A. costaricensis, a tree that exhibits delayed greening and red flushing, to test the difference in herbivory between red and green leaves. We conducted our experiment in January 2013 at Monteverde National Park, Costa Rica.

We haphazardly selected branches containing both red and green compound leaves from 14 A. costaricensis trees. We randomly selected one red and one green compound leaf and then randomly collected three red and three green leaflets to test for herbivory damage, toughness, and glucose. The rest of the leaflets from the two compound leaves were saved for a separate herbivory experiment. We took a photo of the canopy from the site of branch removal, and used imageJ software (Rasband 1997) to calculate percent canopy cover in that part of the forest.

After collection, we traced the leaflets onto grid paper. To measure herbivore activity, we calculated percent leaflet damage by approximating total area of the leaflet and the area eaten. If a leaflet was damaged, we extrapolated total size based on leaflet morphology and comparison with similar leaflets. We used a Chatillon Type 516 penetrometer to measure toughness at a point halfway between the base and the tip of the leaf and between the central vein and the lateral margin. We used leaflet toughness as a proxy for structural herbivore defense (Lucas et al. 2000).

To measure leaflet sugar concentration, we cut a 4 cm² piece from each leaflet and crushed it in a microcentrifuge tube, adding one drop of water from a pipette. If leaflets were smaller than 4 cm², we combined leaflets of the same tree and color to attain an equal amount of leaf matter in all replicates. We ground the leaflet and water mixture to a pulp and measured sugar concentration of the extract solution using a Brix refractometer.

We used the rest of the collected leaves to test herbivore preference. We traced 36 leaflets, three red and three green, to be fed to seven generalist herbivores from the order Phasmatodea (stick

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Means ± SE</th>
<th>Means ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference % Eaten</td>
<td>4.41 ± 1.57</td>
<td>16.46 ± 3.5</td>
</tr>
<tr>
<td>Difference in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toughness (g)</td>
<td>118 ± 24.2</td>
<td>205 ± 17.1</td>
</tr>
<tr>
<td>Difference in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>refractometer reading</td>
<td>6.5 ± 0.803</td>
<td>4.1 ± 0.348</td>
</tr>
</tbody>
</table>
insects). We placed each insect in a separate container (four butterfly nets and three glass tanks) with six similarly-sized leaflets (three red and three green) at the Monteverde Butterfly Garden. We left the walking sticks in the containers overnight and collected the leaflets in the morning. We compared the leaflets collected in the morning to the tracings we made the night before to determine the amount of herbivore damage.

Statistical Analysis
To test for the difference in herbivore damage, leaflet toughness, and sugar concentration between green and red leaflets, we used a series of matched-pairs t-tests. For all analyses, we averaged measurements for each triplet of same-colored leaflets from each tree. To ensure that variation in toughness was not due to leaflet size, we first used regression to test the effect of size on penetrometer reading. We used JMP 10.0 software for all analyses and the assumptions for all statistical analyses were met.

RESULTS
Green leaflets sustained more damage than red leaflets that were collected from the same tree ($t_{13} = 2.946, P = 0.011$; Table 1, Figure 1). Our matched pairs t-test revealed that green leaflets were significantly tougher than red leaflets ($t_{7} = 4.68, P = 0.002$; Table 1, Figure 2) and this result was not confounded by a relationship between leaflet size and toughness (linear regression: $r^2=0.004, P = 0.705$). Sugar concentration (degrees Brix) was significantly higher in red leaflets than in green leaflets ($t_{13} = 2.08, P = 0.058$; Table 1, Figure 3). We found no herbivore damage on any of the leaflets, red or green, presented to the walking sticks.
sticks and these data will not be discussed further. Finally, the trees sampled grew in areas of the forest with an average of 23% light exposure (77% canopy cover).

**DISCUSSION**

We found that flushing red may serve as an anti-herbivore defense mechanism in *Alfaroa costaricensis* as there was significantly less herbivore damage on red leaflets despite a higher sugar concentration and lower toughness. Previous studies have shown that trees translocate sucrose to immature leaves to promote rapid growth and maturation (Turgeon 1989), and that new growth leaves lack the tough outer layer of mature leaves (Choong 1996; Lucas et al. 2000). The lack of structural defense in the form of a tough cuticle, combined with the high sugar content of young red leaves, should render them vulnerable to herbivory, yet we found that red leaflets had a significantly lower percent leaf damage than green leaflets.

We speculate that red coloration makes the leaves appear less appealing to herbivores. Many herbivorous invertebrates are unable to see in the orange-red light spectrum and thus red leaves appear grey and less appealing for consumption (Juniper 1993). Red coloration may also be associated with toxicity (Karageorgou and Manetas 2006), serving as an aposematic signal deterring predation. Repeating our walking stick experiment over a longer period of time could determine whether red leaflets are truly less appealing to herbivores, or if red leaflets are less damaged simply because they are newer growth and have had less exposure to herbivory. To further test the protective role of red leaves, a future study could explore leaf cutter ant preference between red and green leaves to determine if red coloration is an antifungal defense (Coley and Aide 1989). We observed leaf cutter damage on green leaves of Lauraceae and Myrtaceae, which also flush red, but saw little or no damage on red flushed leaves. Leaf cutter ants harvest leaf matter to grow fungi for food and thus preference toward green leaves may suggest an inability of red leaves to grow fungi.

We were unable to account for variation in herbivory exposure time in our analyses, which may have skewed our results. Although green leaves have been exposed to herbivory much longer than new red leaves, 60-80% of lifetime herbivore predation on tropical plants occurs on immature leaves (Coley and Aide 1991; Coley and Barone 1996). Our field observations also indicate that when *A. costaricensis* leaflets reached mature size, they began to green from the base of the leaflet. In leaflets with a gradient of green to red coloration, green areas often exhibited herbivore damage while the red tip remained fully intact, suggesting that that red coloration deters herbivory.

Our findings and observations lead us to speculate that *A. costaricensis* is able to delay greening in part because it grows in light-rich environments where it can afford to limit photosynthesis to protect its new leaves. While we did not specifically test the hypothesis that red leaflets increase the shade tolerance of a plant, our study suggests that red coloration is not necessarily related to shade adaptation since *A. costaricensis* is a relatively shade intolerant species (Arnaea and Moreira 2002). We observed that *A. costaricensis* grew into the canopy and continued to flush red at all heights. Although further study is necessary to quantify how much productivity is lost due to delayed greening, our study implies that limiting photosynthetic capability in favor of herbivore defenses is beneficial to a plant when light is not a major limiting factor. The ability to defend new growth against herbivory may explain the success of *A. costaricensis* as a light gap colonizer. Species race for limited space in light gaps, thus adaptations to maximize growth and minimize loss to herbivory are vital to outcompeting other successional species.
ACKNOWLEDGEMENTS
We would like to thank the staff of Monteverde National Park for providing accommodation and sustenance, Frank Joyce for his guidance and advice, the staff of the Monteverde Butterfly Garden for access to their facilities and walking stick insects, Z. Gezon and R. Chavez-Ulloa for assisting with experimental setup, and R. Calsbeek for his general guidance and consultation.

AUTHOR CONTRIBUTION
All authors contributed equally.

LITERATURE CITED


THE EFFECT OF ANTHROPOGENIC INPUTS ON BENTHIC STREAM INVERTEBRATES IN A TROPICAL MONTANE STREAM

JIMENA DIAZ, SAMANTHA C. DOWDELL, VICTORIA D. H. STEIN

Faculty Editor: Ryan Calsbeek

Abstract: Anthropogenic inputs to streams can severely impact benthic macroinvertebrate (BMI) communities. BMI community abundance and diversity often decrease with increasing human influence and therefore function as an indicator of watershed health. We sampled BMI abundance and community structure along an elevational and anthropogenic gradient in the Quebrada Máquina in Monteverde, Costa Rica. We sampled BMI at nine different sites; four were located above the town of Monteverde, and five were located throughout town. Our results demonstrated that benthic macroinvertebrate abundances for all functional feeding groups decreased with decreasing elevation, while order evenness and richness remained constant among sites. Furthermore, dissolved oxygen (measured as percent saturation), which is a potential indicator of anthropogenic influence, increased with increasing elevation. Accumulated anthropogenic input may have decreased dissolved oxygen levels, thereby effectively decreasing the BMI carrying capacity of the stream length. The reduced abundance of BMI with decreasing elevation in Quebrada Maquina is both an indicator of poor watershed health and a driver of further ecological change.

Key words: benthic macroinvertebrates, cloud forest, elevation gradient, neotropical stream

INTRODUCTION

Watersheds impact human and natural communities in innumerable ways, providing a wealth of resources and services to their environments. Benthic macroinvertebrates (BMI) have often been used as effective bioindicators of stream health, allowing researchers to determine stream water quality, food web dynamics, and possible anthropogenic impacts on watersheds (Karr 1999, Jacobsen et al. 2008). Abiotic factors such as temperature, elevation, pH, and dissolved oxygen can interact with one another and alter BMI community composition (Jacobsen et al. 2008). Dissolved oxygen content of stream water, in particular, has been used to predict water quality, and is sensitive to anthropogenic inputs (University of Wisconsin 2003).

The Quebrada Maquina, a tropical montane stream near the town of Monteverde, Costa Rica flows down an elevational and anthropogenic gradient. The stream begins above town and passes by residential and commercial areas. The effects of anthropogenic input in Quebrada Maquina have been documented, but previous research lacks a baseline investigation into the BMI community upstream of the town.

Although the effects of human input on stream health measured by dissolved oxygen levels and BMI communities have been well-documented (State of Washington Department of Ecology), there is no real consensus on how benthic macroinvertebrate taxa richness and abundance vary with elevation (Henriques-Oliveira and Nessimian 2010). As such, we predicted that BMI abundance would decrease along a decreasing elevation gradient. We also predicted that sites sampled above town would have a greater abundance of macroinvertebrates than sites sampled throughout the town because of the increasing anthropogenic influence.

METHODS

We tested the effects of elevation and abiotic factors on BMI abundance in a neotropical montane stream. We conducted our experiment on January 22 and 23, 2013 in the Quebrada Máquina, Monteverde, Costa Rica. We sampled the Quebrada Máquina at nine sites, following an elevation gradient. We selected sites haphazardly based on accessibility to the stream. At each site, we estimated percent canopy cover and used a Garmin GPSmap 76CSx to record latitude, longitude, and elevation. We sampled three flow types at each site: pool, riffle, and run. For each flow type, we recorded dissolved oxygen percent saturation and temperature using a YSI Digital Professional Series ProODO. We sampled BMI at each flow type by agitating the sediment upstream.
of an aquatic net for one minute and transferring net contents into a Ziploc bag. We collected a total of 27 samples.

In the laboratory, we filtered the contents of each Ziploc through three sieves (4000, 2000, and 500 microns). We sorted through each sieve for macroinvertebrates visible to the naked eye (Jacobsen et al. 2008). We counted and identified each macroinvertebrate to order and functional feeding group (shredder, grazer, collector, or predator). In some cases functional feeding group could only be determined by identifying the macroinvertebrate to family.

**Statistical Analysis**

We tested total macroinvertebrate abundance against percent dissolved oxygen (%DO), elevation and temperature using multiple regression. We then used correlation to test whether %DO varied with elevation. We used a non-parametric, Spearman Rank Correlation to test individual feeding group abundances against elevation. We also tested whether the abiotic factors we measured (percentage canopy cover and temperature) were correlated with elevation. We used the vegan package in R to calculate Simpson’s Diversity Index and richness rarefaction to test whether macroinvertebrate order evenness and richness changed over our elevation gradient (R Development Core Team 2011). We log10 transformed total macroinvertebrate abundance to ensure that our data met the assumptions of the statistical tests used. We performed all analyses using JMP 10.0 software.

**RESULTS**

Dissolved oxygen percent saturation (%DO) was the most significant factor driving total macroinvertebrate abundance. The full multiple regression model showed that DO percent saturation explained more variation in total macroinvertebrate abundance than elevation ($r^2 = 0.73$, $F_{2,26} = 32.87$, $P = 0.0021$, Figure 1). Elevation and %DO were multicollinear and mean DO percent saturation was higher in high elevation sites ($\bar{x}_{\text{high}} = 85.79 \pm 0.52$) than low elevation sites ($\bar{x}_{\text{low}} = 80.92 \pm 0.47$; Mean ± 1SE, $r^2 = 0.66$, $P < 0.0001$, Figure 2). We also found that individual macroinvertebrate feeding groups were negatively correlated with elevation (Table 1).

Percent canopy cover did not differ significantly with increasing elevation ($r^2 = 0.06$, $P = 0.22$). However, mean temperature was significantly different among high ($\bar{x}_{\text{high}} = 16.63 \pm 0.06$) and low elevation sites ($\bar{x}_{\text{low}} = 17.44 \pm 0.06$; Mean ± 1SE, $r^2 = 0.78$, $P < 0.0001$).

Order richness and evenness remained the same along our elevation gradient. We found that macroinvertebrate order rarefied richness did not differ significantly among sites on a decreasing elevation gradient ($r^2 = 0.13$, $P = 0.34$, Figure 3). Order evenness did not differ significantly among
sites on a decreasing elevation gradient ($r^2 = 0.009$, $P = 0.79$, Figure 4).

DISCUSSION

Our results suggest that the decline in total macroinvertebrate abundance along the Quebrada Maquina is driven by anthropogenic influence. Our elevational gradient was a proxy for an anthropogenic gradient rather than two distinct systems (unaffected or affected by humans), because human impact on the stream accumulates along the stream’s run. Although the higher elevation sites were located above downtown Monteverde, they were not completely free of anthropogenic disturbance. Monteverde’s Biological Station is located above our highest-elevation study sites and roadways leading to the station are used on a daily basis. Trash was visible throughout the lower reaches that we sampled, and we observed other anthropogenic influences including automobile use, agriculture and livestock grazing in the town itself.

The difference in percent dissolved oxygen saturation between high- and low-elevation sites could be explained by human input because the addition of chemical and organic pollutants increases oxygen demand and decreases the system’s biotic carrying capacity (State of Washington Department of Ecology). Boulton et al. (1997) demonstrated that although macroinvertebrates may be able to survive in dissolved oxygen percent saturations as low as 20%, both taxa richness and total abundance increased with dissolved oxygen percent saturation. Therefore, the observed decrease in macroinvertebrate abundance may be the result of decreased dissolved oxygen caused by local human activity.

The other abiotic factors we measured did not appear to influence BMI abundance. Mean temperature decreased from high elevation sites to low elevation sites. However, the magnitude of difference was only one degree celsius, which has proven insufficient to impact BMI assemblages in streams (Durance and Ormerod 2009). Similarly, canopy cover did not explain changes in BMI abundances because there was no significant variation between sites.

The diversity and abundance of the BMI community of the Quebrada Máquina could have severe effects on the surrounding tropical cloud forest. Streams and headwaters are key components of watershed health, increasing resource flows both up and downstream and in and out of the water (Allan 2004). Allochthonous inputs are decomposed, recycled and deposited back onto the land. Meanwhile, some stream insects (aquatic larval forms and terrestrial adults) facilitate the movement of nutrients and possibly pollutants in and out of the water, fundamentally impacting food webs and chemical interactions well away from the waterway (March and Pringle 2003).

Further research is needed to determine the exact chemical or physical anthropogenic input in the Quebrada Máquina to more precisely pinpoint possible contaminating agents. The macroinvertebrate watershed as a whole, including the lower reaches of the Quebrada Máquina that flow into the Río Guacimal, should be tested for contamination as well. The reduced abundance of
BMI in the lower reaches of the Quebrada Maquina is both an indicator of poor watershed health and a driver of further ecological change.

ACKNOWLEDGEMENTS
We would like to thank Ramsa Chaves-Ulloa and Zachariah Gezon for their guidance in project development and stream location. We would also like to thank the staff of the Monteverde Butterfly Garden for providing us with directions to locate Quebrada Máquina past downtown Monteverde and the local man who allowed us access to the portion of the stream on his property.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED


OCCUPATING SMALL-SCALE TROUT FARMING: EFFECTS OF TAGGING, DIET, AND WATER QUALITY ON *ONCORYNCHUS MYKISS*

AMELIA F. ANTRIM, SETH A. BROWN, SAMANTHA C. DOWDELL, MARIA ISABEL REGINA D. FRANCISCO, AND MOLLY R. PUGH

Faculty Editor: Matthew Ayres

**Abstract:** Conditions such as population density affect growth and reproduction of organisms. Aquacultural operations attempt to optimize these conditions to maximize profits. To balance the biological tradeoffs inherent in trout farming, farmers can monitor water quality and track the growth and reproduction rates of successful reproductive females using fish tags. We tested the effects of diet on body condition and the effects of fish tags on intraspecific interactions, specifically the risk of aggression. Tags did not result in increased aggression between fish and appear to be a safe and effective way to monitor fish condition and growth rate. The body condition of trout-eating females was higher than that of pellet-eating females, indicating the high-protein, constantly-available food increases fish growth and market value. Because trout require high-oxygen environments, we measured dissolved oxygen (DO) in all pools as a determinant of water quality. DO levels were highest in the areas that were exposed to wind and that had smaller surface-area-to-volume ratios. Understanding the biology behind methods such as fish tagging, food optimization, and water quality monitoring allow farmers to maximize the value of their stock.

**Key words:** aquaculture, body condition, *Oncorhynchus mykiss*, tagging

**INTRODUCTION**

Population density and environmental conditions affect rates of growth and reproduction in individuals (Moyle et al. 1996, Jenkins et al. 1999). Humans can artificially manipulate stock densities as well as food and holding conditions to optimize growth and reproduction. Industries that manage populations for economic gain must balance the tradeoff between growth rates and stock size. Additionally, maintaining a breeding population might involve artificially selecting for individuals with desired traits and therefore altering the population.

Aquacultural operations can optimize population management and selective breeding by tagging stock and monitoring individuals’ success. Tagging is beneficial on trout farms, where farmers must cultivate large populations while keeping track of individuals. However, trout tagging is mildly invasive in that it punctures the dorsal fin, increasing drag and potentially altering swimming ability. Additionally, conspicuous tags may inadvertently cause fish to harm one another because trout are curious about potential food items and display high rates of intraspecific aggression (Ellis et al. 2002, Solano pers comm. 2013). Trout farmers that previously did not use fish tags may therefore be wary of introducing them, and might be reassured by empirical research on their effects.

Additionally, farmers may maximize productivity in trout farms by optimizing the tradeoff between food quality and price. Some trout farms may opt to feed their trout inexpensive commercial pellets on a regimented schedule rather than protein-rich live prey. However, providing trout with higher dietary protein and a higher caloric intake may increase body mass, making them more economically valuable (Lee and Putnam 1973). Farmers may therefore benefit from investing in higher quality food.

Trout farming can be a profitable livelihood in developing areas of the tropics, despite the fact that trout are non-native to these regions (Gurung and Basnet 2003). Tropical trout farms must develop techniques for
increasing the quality of their stock under local conditions (Gurung and Basnet 2003). We tested techniques for optimizing sustainable aquaculture at Cuericí Biological Station, a trout farm in Costa Rica. We assessed methods for improving trout stock by testing the effects of tagging on rainbow trout, specifically comparing intraspecific aggression toward tagged fish and untagged fish. If tags are highly conspicuous to trout, tagged fish would be more frequent targets of intraspecific aggression.

Farmers can benefit from tracking growth rates and water quality to assess trout growth and reproduction over time. For example, farmers can keep track of the most reproductively successful females by periodically taking length and weight measurements of tagged fish. We tagged and measured fish to facilitate future studies of the growth rates of known individuals. We also recorded water quality measurements and collated previous measurements to facilitate tracking of water quality across years. Finally, we assessed the benefits of a protein-rich, high-calorie diet by comparing the mass and morphology of reproductive female fish who had been feeding continuously on other small trout with females of a similar length who were being fed fish food.

METHODS

We conducted our study from January 30 to February 1, 2013, at Cuericí Biological Station, located at 2500 m/asl near Cerro de la Muerte, Cartago, Costa Rica. The station primarily consists of a small-scale farm, including a rainbow trout hatchery. The farm employs natural methods for optimizing trout growth and reproduction: fish are hatched and grown using stream water that is not treated or artificially oxygenated, and are not given antibiotics. Using two large nets, we collected 42 female rainbow trout from pools 11, 12, and 13 (Figure 1). Only female fish were used for trials because female fish are most valuable to tag and track for reproductive success. We measured the length of each focal fish using a tape measure and weighed the fish on a hanging balance. Fish were then randomly assigned to one of four treatments: untagged all-female (placed in a tank with four other females), untagged mixed (placed in a tank with two males and two females), tagged all-female, or tagged mixed. All non-focal fish were haphazardly chosen from pools 11, 12, and 13.

After weighing and measuring each fish, we placed the fish in a small holding tank. Fish assigned to tagging treatment were tagged using the Avery Dennison TM Mark III pistol grip tool and orange Floy® Tag & Mfg., Inc., tags at the base of the dorsal fin. We allowed the fish to recover in the holding tank, then transferred the fish to the tank containing all-female or mixed fish and observed the fish for two minutes, recording contact with and attacks from other fish. Attacks were defined as attempts to bite, while contact was defined as any bodily contact between fish. We then removed the fish from the tank and returned it to pool 10, 11, 12 or 13; we did not necessarily return fish to their original pools as pools 10-13 contained the same age classes of fish.

To collect preliminary data for future measurements of reproductive success, we tagged the reproductive females in pool 14, henceforth referred to as “pellet-eating females” as they were fed commercial fish pellets and occasionally worms. We recorded their weight and length, and moved them to pool 16. In addition, we assessed water quality by measuring dissolved oxygen (DO, mg/L), temperature, and pH of all pools using the map and sites from Wengert et al. 2009 (Figure 1). We collated these data for comparison with previous and future Dartmouth studies.

Finally, to assess the effect of high-quality food on fish growth, we captured and tagged reproductive females that had escaped from pool
14 into pool 15 prior to the experiment. These were called “trout-eating reproductive females” because the owner observed that they had been feeding on the smaller trout from pool 15. We weighed, measured, and tagged these fish before transferring them to pool 16.

Statistical analyses
All analyses were conducted using JMP 10.0 statistical software and assumptions for all tests were met. To determine whether tagging increased aggression towards the focal fish, we performed a t-test comparing the total number of contacts with other fish between tagging treatments.

To investigate how diet affects fish mass, we calculated body condition as the residuals of a regression of mass vs length and used a t-test to compare pellet-eating and trout-eating females of about the same length (52-57 cm).

We compared dissolved oxygen concentrations among groups of pools with different age classes of fish: pools 5, 6, and 7 were younger hatchery; 8 and 9 were older hatchery; 10, 11, 12, and 13 were troughs; and 14, 15, and 16 were breeding pools.

RESULTS
We did not observe any attacks between fish throughout the course of our study. Also, we found no difference in the number of times the focal trout came into contact with other trout between tagged and untagged trout (t=0.71, df=38, P=1.00, Figure 2).

Trout body condition varied with diet. Body condition of the trout-eating reproductive females was significantly higher than pellet-eating reproductive females of similar length (x̄ ±1SE: 0.25±0.08 and -0.10±0.04 respectively; t=3.91, df=15, P=0.001, Figure 3).
Dissolved oxygen varied among pool groups (Figure 4). It was highest in the older hatchery and troughs than in the breeding pools and younger hatcheries. Maximum DO of all the pools was measured in the older hatchery at 7.85 mg/L, while minimum DO was measured in the younger hatchery at 4.41 mg/L.

Temperature varied from 12.2 to 15 °C, while pH varied from 6.65 to 7.41.

**DISCUSSION**

As fish did not react differently toward tagged fish, we saw no risk in tagging farmed trout. We recommend tagging female trout so that farmers can keep track of individuals. Operations can then identify high quality breeding females and track rates of growth and reproduction among individual fish.

The body condition of the trout-eating reproductive females was greater than that of the pellet-eating reproductive females. The trout-eating females consumed smaller trout, giving them constant access to a high-protein food source in addition to the typical pellet food. The pellet-eating females had no access to smaller trout and were only able to eat when provided with pellets. While high quantities of protein-rich food may be expensive, a diet higher in protein and caloric value may cause body mass to increase, thus raising the market value of the fish (Lee and Putnam 1973). Larger females also produce larger eggs and juveniles (Ojanguren et al. 1996), increasing their long-term value. Further studies are needed to determine the cost-benefit tradeoff of providing trout with high-quality food.

Monitoring dissolved oxygen levels is critical for the productivity of trout farms, as *O. mykiss* growth rates decrease when DO falls below 5 mg/L, and fish suffocate when levels fall below 4 mg/L (Molony 2001). On the Cuericí trout farm, the older hatchery and troughs both had relatively high DO levels, while the breeding pools had moderate levels, and the younger hatchery had relatively low DO levels compared to the other pools. The older hatchery may have had higher DO levels than the younger hatchery because it is located outside, allowing wind to mix the pools’ surface (Craggs et al. 2013). The troughs may have had higher DO levels because they contain many...
photosynthetic plants and algae that produce extra oxygen (Chang and Ouyang 1988). Conversely, the low surface-area-to-volume ratio in the large, round breeding pools may result in decreased wind mixing (Craggs et al. 2013). Therefore, the shape of the breeding pools may contribute to their moderate DO levels despite the presence of photosynthetic plants and exposure to wind. The younger hatchery could have had low DO levels due to the high density of fish and the enclosed location of the troughs, preventing mixing by wind and the growth of photosynthetic plants or algae (Chang and Ouyang 1988, Craggs et al. 2013). Although DO varied between locations, all measurements, except two taken within the younger hatchery, were above the critical low DO concentration for *O. mykiss*. We recommend continued monitoring of DO and study of factors that influence DO.

The presence of individually marked fish will facilitate future studies, such as how current body size affects future growth. Intraspecific competition in fish populations frequently results in increased disparities in size, as larger fish may outcompete smaller fish for food, and thus grow more quickly than their competitors (Cuenco et al. 1985). If intraspecific competition does lead to unequal growth rates, we recommend that small and large fish be segregated to equalize growth rates.

Methods such as fish tagging and food optimization allow farmers to monitor and increase the value of their product, helping small-scale farms to compete with larger aquacultural operations. Tradeoffs such as the ones examined in this study must be favorably balanced. An understanding of the biological and environmental factors that influence growth and reproduction in trout and other agricultural stock is crucial to the success and advancement of agriculture.

ACKNOWLEDGEMENTS
We thank Don Carlos for giving us permission to work with his trout and for sharing with us his knowledge of natural and sustainable trout farming.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED


Molony, B.. 2001. Environmental requirements and tolerances of rainbow trout (Oncorhynchus mykiss) and brown trout (Salmo trutta) with special reference to Western Australia: a review. Department of Fisheries, Government of Western Australia.


Solano, Carlos. Personal communication, January 2013.

FLIES AND FLOWERS: INVESTIGATION OF FLY AGGREGATIONS WITHIN *N. speciosa* FLOWERS

TYLER E. BILLIPP, COLLEEN C. COWDERY, AND VICTORIA D. STEIN

Faculty Advisor: Matt Ayres

Abstract: Biotic and abiotic factors influence animal dispersion as the animals seek to fill their environmental, reproductive, and foraging needs. A species of fly has been observed to be highly aggregated in the flowers of *N. speciosa*; these flies might gather in flowers for thermal benefits, mating, or foraging opportunities. To explore possible causes behind fly distribution among flowers, we measured aggregation, temperature variation in the flowers over time, sex ratio of flies within flowers, evenness of distribution between flowers, and flower style length. The fly aggregations were not driven by thermal benefits, mating behavior, or habitat for larvae. However, all large aggregations of flies occurred in flowers with style lengths between approximately 20 and 35 mm. This stage of flower development coincides with young, male flowers with copious pollen, which might be a food resource for the flies. Spatial patterns in *N. speciosa* and its inhabitant flies provide insight into the general factors that influence spatial dispersion of animals across a landscape.

Keywords: animal dispersion, animal-plant interactions, *N. speciosa*

INTRODUCTION

Interactions among biotic and abiotic factors affect the dispersion of organisms across a landscape. Animals distribute themselves in complex patterns across space due to thermoregulatory requirements, mating opportunities, random dispersion, and energy and nutrient availability, among other factors (Connell 1963, Gautestad and Mysterud 2004). Understanding patterns of dispersion at large and small scales has basic value and can be relevant to applied ecology, for example conservation biology.

One type of dispersion pattern, aggregations, is common among animals in nature (Parrish and Edelstein-Keshet 1999). Aggregations can be indicators of resource distributions or habitat suitability. Aggregation can be the result of thermoregulatory demands influencing the dispersion of poikilothermic and ectothermic animals, which use their surroundings as either heat sources or heat sinks (Lillywhite 1970, Huey 1991). These organisms should aggregate in areas of locally extreme temperatures to maximize heat exchange in shorter time, which would allow more time for foraging and reproduction (Bowker and Johnson 1980). Animal aggregations may also reflect dispersion based on mating resources, as in male lekking (Jarvis and Rutledge 1992) or male territoriality (Fellers 1979). Depending on which sex is the “choosy” sex and the differential survival of each, reproductive aggregations should be evident from the ratio of one sex to the other. For example, female-to-male sex ratios in reproductive aggregations of some meloid beetles are 1:8 (Snead and Alcock 1985). A 1:1 sex ratio would instead imply random assortment of flies within flowers. Whatever the ratio, if it is driven by reproductive aggregation behaviors, one would expect it to be relatively constant among all suitable lekking locations, such as within the flowers of *N. speciosa*. Aggregation for foraging purposes, according to optimal foraging theory, should result in an equal ratio of user to resource, regardless of patch quality (Charnov 1976).
Accordingly, when resources are randomly or evenly distributed, animals should spread evenly across the landscape.

To study factors affecting aggregation in animals, we studied adult flies (Diptera) that aggregate inside the bell-shaped flowers of *Nasa speciosa* (Loasaceae). Although Jennings et al. (2000) observed these fly aggregations and hypothesized that the poikilothermic flies used the flowers as miniature greenhouses in cool mornings to gain temperature and therefore foraging advantage, our field observations and examination of their data pointed to alternative possibilities. We observed flowers in which flies were highly aggregated with little apparent temperature bias, which led us to develop several competing hypotheses: thermal benefits as hypothesized by Jennings et al. (2000), mating, or foraging. The first hypothesis predicts that flies would aggregate in warmer flowers versus cooler, shaded flowers. The second hypothesis predicts some patterning in sex ratio, and would be supported by observations of mating in flowers. If flies are using the flowers to forage, we would predict a uniform dispersion of flies among flowers to minimize competition for nectar.

**METHODS**

We studied four patches of *N. speciosa* at Cuericí Biological Reserve, Costa Rica from January 30 to February 1, 2013. The patches were located within 1.3 km of each other along a canyon next to the Cuericí trail. We located 52 flowers total, collected 38, and obtained complete data sets for 32. For the 38 flowers we collected, we counted and sexed all flies present in each flower.

We outfitted five haphazardly-chosen focal flowers from one patch with thermocouples for approximately 24 hours from the morning of Jan. 31 to Feb 1 to measure the thermal characteristics of the flowers. We measured both ambient air temperature and internal flower temperature every ten minutes using Hoboware Data Logger JKST thermocouples. After temperature data were collected, all 13 flowers in the patch (including the 5 thermocoupled flowers) were collected with whatever flies they contained.

To obtain a wider flower temperature data set, we used a handheld Raytek Raynger MX infrared sensor to measure the internal and ambient temperature of 15 haphazardly-chosen flowers from 8:00-9:00 am on Feb. 1. The flies and flowers were subsequently collected.

To determine if the flies were foraging in the flowers versus mating, we observed fly behavior, arrivals and departures, and total abundance with 1 minute observation trials every 10 minutes. On Jan. 30, we performed observation trials on 6 flowers from 7:30-9:30 am; on Jan. 31 we observed 4 flowers from 8:30-9:15 am. All flowers and flies were collected post observation for both days for a total of 10 flowers.

To investigate possible protandry in *N. speciosa*, we collected and measured styles from every flower from Jan. 31-Feb. 1. We measured style length on all 32 flowers collected for those 2 days. We also dissected each flower from Jan. 31-Feb.1 to search for fly adults, larvae, or eggs hidden behind nectaries.

**Statistical Analyses and Modeling**

We compared the probability of finding our observed fly distributions against a Poisson discrete probability distribution, representing the null hypothesis of a random dispersion (999 iterations using a Monte Carlo simulation created in R; R Core Development Team 2011). We
compared temperature variations and total fly abundance per flower. We tested for a correlation between fly sex ratios and fly abundance per flower. We also tested the relationship between style length and total fly abundance. Analyses were performed with JMP 10.0 statistical software; after verifying that assumptions were met.

**RESULTS**

The 53 *N. speciosa* flowers contained from 0 to 430 flies. We found 28 flowers containing no flies, while the other 25 flowers contained on average 78.0 flies (SE = 22.4). The Monte Carlo randomization tests indicated that the flies were highly aggregated (p < 0.001).

Air temperature within the five thermocoupled flowers was warmer than ambient air temperature from 10:50 am to 3:50 pm and otherwise matched ambient air temperatures, but flies did not aggregate in the flowers that reached the highest temperature (Fig. 1). In the 15 flowers collected on Feb 1, flies were also aggregated irrespective of flower temperature; the coolest flower (8.5°C) actually contained the most flies (430 individuals).

We found a total of 1085 female flies and 864 male flies in all flowers combined. This was a significant deviation from 1:1, (p < 0.0001), but the female bias was about the same regardless of whether flowers had few or many flies in total (data not shown). Additionally, no mating behavior was noted during observation trials.

Flower developmental stage seemed to affect fly aggregation. We found a strong relationship between fly aggregations and flowers that possessed styles ranging from approximately 20-35mm in length (Fig. 2). Styles were longer in flowers that appeared older, with more damage and less remaining pollen; buds and newly opened flowers had shorter style lengths.
In the 24 hours of combined observation time, we recorded three hummingbird visits *N. speciosa* flowers. A magnificent hummingbird (*Eugenes fulgens*) and two other unidentified hummingbirds visited several flowers each, apparently exhibiting traplining foraging behavior.

**DISCUSSION**

Our data dis not support the hypothesis that flies aggregate within *Nasa speciosa* for thermal benefits. We did not find a meaningful thermal difference between the flowers and ambient air during the night or early morning, suggesting fly aggregation in flowers during those times did not offer thermoregulatory benefits. There were temperature differences at midday or while the flowers were in direct sunlight, but since those occurrences coincided with peak ambient temperatures outside the flowers, it seems that the solar warming of flowers did not provide any meaningful advantage to the flies. Additionally, no flies were observed leaving or entering flowers during our observations in the mornings, as one would expect if the flowers were being utilized only in behavioral thermoregulation during cold periods, and not for fly foraging. Flies might choose flowers in which to aggregate in the evening (since no movement was observed in the morning), but this still does not suggest thermal benefits because flowers were essentially at ambient air temperature throughout the evening and night.

We found little support for mating-driven fly aggregation. No mating or courtship behaviors were observed during the study, though we cannot rule out their occurrence without more rigorous behavioral observation. While generally female flies were more abundant than male flies, there was no evidence for leks or other structure to the sex ratios among flowers. Additionally, our observations
and flower dissections did not reveal any sign of eggs or larvae within the flowers, implying that the flowers are not serving as hosts for young flies.

Our results best supported the resource acquisition theory of fly aggregation in *N. speciosa*. While the massive aggregations of several hundred flies did not appear to be in line with optimal foraging theory given the presence of so many empty flowers, Sutherland (1983) found that interference within an aggregation of foragers in fact stabilized optimal foraging models, such that aggregations of resource users within a single patch could still be optimal. Additionally, all fly aggregations occurred in flowers with a narrow range of style lengths. The striking developmental variability in style length (short in new buds and newly opened flowers, long on older-looking flowers and fruits) suggests protandry in *N. speciosa*. If the flies are targeting a specific developmental stage of the flower, the aggregations could coincide with the availability of pollen, which would increase the chances that the flies are foraging optimally since only those flowers would possess the desired resource. We never observed flies consuming pollen or nectar, but it would have been difficult to see. Flies were never observed to leave the flowers to forage elsewhere. Further study of the life history of *N. speciosa* and Loasaceae in general would be helpful in unraveling this apparent developmental association. While we did not collect meaningful data on nectar or pollen quantity within the various flowers, observation of multiple hummingbird visitations suggests that nectar is present. Future studies might examine the relationship of the flies to nectar and pollen and test for the existence of a plant-pollinator mutualism or a pollen and nectar predation relationship.

An alternate explanation for fly aggregation might be that flies are deriving some shelter or antipredator benefit from the flower’s umbrella of petals and the urticating hair defenses on the plant. Aggregation has also been shown to confer net fitness advantage to insect pupae by decreasing predation hazard (Wrona and Jamieson Dixon 1991). However, we discounted this hypothesis early in our observations because urticating hairs would deter large predators such as mammals but probably not predators of a size likely to prey on our species of studied Diptera. It is still possible that the flowers provide protection by allowing the flies to hide from visual predators, as we observed the flies to be spending the majority of their time deep within the flower, but this does not explain the fly preference for a certain developmental stage of flower.

Pollenivory or nectivory in these flies might be an example of plant-insect mutualisms if the flies are also pollinating the flowers. However, since none of the flies we collected carried any pollen, they are probably more analogous to birds or squirrels who seek out ephemerally fruiting plants. Future studies could investigate the phenology of this relationship if plant and fly populations fluctuate seasonally. This type of resource heterogeneity can be of general importance in defining the distribution of organisms and their relationships to the habitats they occupy.

ACKNOWLEDGEMENTS
Special thanks to Zachariah Gezon for help with R and the Monte Carlo simulation, Don Carlos Solano for his natural history contributions and advice, and Gillian Britton, Liza Huntington, Kali Pruss, and Emilia Hull for help in locating our study system.
AUTHOR CONTRIBUTIONS
T. Billipp made initial observations, V. Stein contributed identifications and natural history research, and C. Cowdery illustrated the studied flies. All authors contributed equally to data analysis and writing.

REFERENCES
R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for
EFFECT OF FISH DENSITY ON METABOLISM OF *ONCORHYNCHUS MYKISS* FRY

JIMENA DIAZ, ELLEN T. IRWIN, AND ELISABETH R. SEYFERTH

Faculty Editor: Matt Ayres

Abstract: Population density influences the health, reproduction, and survival of organisms. For example, organisms at higher population densities may experience increased metabolic rates and thus decreased food conversion efficiency. Farmed species in particular are often raised at high densities to increase total production. However, maintaining the health and growth rates of farmed organisms while increasing economic returns can often represent a trade-off for farmers. To investigate the effects of fish stocking density on metabolism, we examined the relationship between density and per capita oxygen consumption in rainbow trout fry, *Oncorhynchus mykiss*. Contrary to our expectations per capita oxygen consumption in trout fry decreased with stocking density. These results suggest a possible predator defense strategy, especially as we found that the fry tended to aggregate naturally and aggregation tended to increase when exposed to a predator simulation. While further research is needed on other density-dependent factors that can negatively affect growth and survival, increasing farmed fish density may reduce energy expended through respiration and may increase overall production. The physiological effects of density can be consequential for the productivity and efficiency both agricultural and natural systems.

Key words: Aggregation, aquaculture, optimal stocking density, Oncorhynchus mykiss

INTRODUCTION
Population density influences the health, reproduction, and survival of individuals in a community. For example, density of individuals has been shown to impact disease spread, intraspecific competition for limited resources such as food and reproductive opportunities, and parasitism (Haldane 1956; Tanner 1966). Density can also influence physiological processes such as metabolic rate. In species such as broiler hens, increasing density increases metabolic rate, decreasing food conversion efficiency (Dozier et al. 2006). Density-dependent metabolism holds important implications for growth and development; as metabolism increases, the efficiency of production of biomass per individual decreases.

Consideration of density-dependent metabolic effects is especially important in agricultural systems in which density is artificially increased to produce a higher number of organisms and maximize economic returns. Density plays an important role in the management of tree stands and cattle pastures because managers must consider the trade-off between higher stocking densities and growth rates (Drew and Flewelling 1979). Similarly, farmed fish are often raised at high densities to maximize production and profits. High stocking densities may negatively affect the welfare of farmed fish, causing reductions in food assimilation efficiency and growth and an increase in mortality (Montero et al. 1999; Ellis et al. 2002). In addition, high stocking can produce chronic stress, leading to increased energy demand (Montero et al. 1999). Perhaps due to this, fish raised at higher densities often have lower growth rates and final mass than those raised at lower densities (Irwin et al. 1999). Stress may increase fish oxygen consumption (Barton and Schreck 1987), thus increasing metabolic costs and leading to greater food intake to maintain production. Given such negative effects, it could be more economically favorable to reduce stocking densities to increase growth and minimize food consumption costs. The optimal stocking density, in terms of
economic returns is that which maximizes the production of fish and fish size.

To investigate the effects of fish stocking density on metabolism and therefore food consumption, we examined the relationship between density and per capita oxygen consumption in rainbow trout fry (*Oncorhynchus mykiss*). If per capita oxygen consumption increases with density as fish become more stressed, then the reduction of fish density could increase production without a corresponding increase in food consumption by trout and would therefore allow aquaculturalists to increase economic returns.

**METHODS**

We conducted this study on January 29-31, 2013, at Cuerici Biological Station, Cerro de la Muerte, Costa Rica, using rainbow trout fry (*Oncorhynchus mykiss*). Mean size and mass of focal fish was 50 ± 8 mm and 1.36 ± 0.62 g respectively (mean ± 1 SD). To test how fish density affected per capita oxygen consumption we manipulated density in an approximately 7 L open-topped tank. We randomly selected fish densities from 10 to 99 fish to be placed in the experimental tank (equivalent to 1 to 14 fish/liter). For each trial, we removed the appropriate number of fish from an aquaculture tank containing approximately 25,000 one-and-a-half-month old trout fry. We transferred fish from their original tank to the 7 L tank. We recorded temperature and dissolved oxygen concentration (DO) in mg/L using a YSI Digital Professional Series ProODO prior to adding the fish. After adding the fish, we recorded DO every minute over a 16 minute trial.

To explore a possible mechanism behind the oxygen consumption results from our density manipulations, we investigated whether fry naturally aggregate when in very low densities, especially in response to predation. We visually divided a 5 x 0.87 x 1 m tank containing ~1522 L of water into five 1 x 1 m sections using rope. We simultaneously placed ten fish from a holding tank in each section (50 total fish) and allowed them to move freely for five minutes. At the end of the five-minute trial, we recorded the number of fish in each section. To simulate potential predation, we held an osprey silhouette (Figure 1) above the testing tank and in two six-second passes, moved the silhouette along the length of the tank. After simulated predation we immediately counted the number of fish in each section using the same methods as before.

![Figure 1. Osprey silhouette for predation simulations.](image)

**Statistical Methods**

To test the relationship between per capita oxygen consumption and fish density, we used simple linear regression. We transformed fish density to meet the assumptions of the analysis. To determine if trout fry naturally aggregate, we tested the ratio of variance:mean fish per section against a probability distribution of expected values under a poisson distribution. Expected values were generated using a Monte Carlo simulation written in R (R Development Core Team 2011, simulation created by Z. Gezon). We used separate analyses for each trial. A variance:mean ratio <1 suggested that fish were uniformly distributed among sections. A variance:mean ratio of 1 suggested fish were distributed throughout sections independently of one another (Poisson distribution). A
Figure 2. Oxygen consumption per fish decreased and began to level off with greater density of *Oncorhynchus mykiss*. Oxygen consumption was measured as the change in dissolved oxygen concentration over a 16 minute trial. The arrow represents the stocking density at which trout were kept normally. The non-linear line represents the log\_10 back transformed predicted y-values for these data. This study was conducted January 29 – 31, 2013, in Cerro de la Muerte, Costa Rica.

Figure 3. Aggregation in *Oncorhynchus mykiss* tended to be higher after simulated predation trials. Aggregation was calculated as the ratio of the variance to the mean number of fish distributed among a tank split into five sections. The line represents a one-to-one relationship indicating no change in aggregation after predator simulations.

RESULTS

Per capita oxygen consumption decreased linearly with log10 transformed fish density ($R^2 = 0.76$, $P < 0.0001$). As density increased above approximately 7.7 fish/L, the rate of decrease in per capita oxygen consumption leveled off (Figure 2).

The ratio of variance over mean was significantly greater than one in all non-predator and simulated-predator aggregation trials. Fish in predator-simulated trials tended to aggregate more than before they were exposed to the predator, but the trend was not statistically significant ($t_{11} = 1.29$, $P = 0.11$, Figure 3). Power analysis suggested that a sample size of 59 would have been required to detect a difference this large if it were real.

DISCUSSION

Contrary to our hypothesis, fish at higher densities had lower per capita oxygen consumption than fish at lower densities. This implies lower metabolic expenses and therefore greater production of fish biomass given the same food consumption. The decrease in respiration was nontrivial: from the smallest density measured to the largest, consumption of oxygen per fish nearly halved. Based on high and low estimates of apparent digestibility (AD) and efficiency of consumption of ingested material (ECI), a doubling in respiration rate would require a 33-75% increase in food consumption to maintain production. Given narrow profit margins in small-scale aquaculture aggregated.

To test the difference in aggregation before and after predator simulation, we performed a paired t-test of variance:mean ratio. We also performed a post hoc power analysis on our paired aggregation data to determine the number of samples needed to detect significant results. Paired t test and power analyses were conducted using JMP 10.0.
operations, a large increase in food costs could result in decreased economic returns.

The stocking density of trout in our study system could be increased to further reduce metabolic rates and decrease consumption. Fish density in troughs was roughly 7.7 fish/L and per capita oxygen consumption averaged approximately 8.8 μg O₂/min according to our measurements. If fish density was reduced by 50% to 3.85 fish/L, respiration would increase by about 25% and would therefore require a proportional increase in food consumption to maintain production while also producing fewer fish overall. A 50% increase in fish density to 11.85 fish/L would result in an approximately 12% decrease in respiration rate, which may still result in substantially reduced energy expenditure per fish while producing more fish overall.

It is important to note that our trials were not conducted in a closed environment; oxygen exchange occurred between the water and the atmosphere. Thus our measurements of oxygen consumption rates represents lower estimates - and more so in the trials with more fish - so we expect that the difference in per capita respiration between low and high densities of fish would be even grater if we had been able to perform measurements in a closed system.

One possible explanation for the relationship between oxygen consumption and density is that the fish were accustomed to being kept at high densities and therefore became agitated at lower densities. Above our mid-range densities, which were similar to the actual density at which the fish were kept, oxygen consumption continued to decrease. Furthermore, we found that trout fry did naturally aggregate when spaced evenly, indicating that they preferred being at higher densities. While adult trout do not exhibit schooling behavior (Newman 1956), it is possible that trout fry may school for protection from predators. At lower densities, each individual trout has a higher probability of being consumed during a predator attack (Mooring and Hart 1992). When exposed to a simulated predator, aggregation tended to increase (though not significantly). Given that the trout were already greatly aggregated before predator simulation, fish grouping among only five sections may not have provided the resolution needed to detect further aggregation. Our results might therefore suggest that aggregation in trout fry is partially a response to the threat of predatory attack, though other factors must also influence aggregation behavior. Future studies could investigate other potential explanations for trout aggregation such as how schooling behavior might affect energy expenditure through improved hydrodynamics between closely spaced fry.

Increasing trout density could increase fish production efficiency by reducing fish metabolic rates. This reinforces existing practices of maintaining high densities in fish production systems. However, high density affects more than metabolic rate and may still negatively impact organisms by reducing growth in other ways and by increasing mortality (Montero et al. 1999; Ellis et al. 2002). Future research should investigate factors such as disease and intraspecific aggression in addition to metabolism, and could further clarify the optimum density for reducing energy expenditure while maintaining a high growth rate that would maximize production.

Density is also an important factor to consider in the management of natural systems. Increasingly smaller habitats place constraints on the growth and survival of populations (Fahrig 2001). Density constraints can affect the distribution, abundance, and health of both ecologically and economically valuable species (Huchette et al. 2003). Therefore, the physiological effects of density must be taken into account in both agricultural and natural systems when working to maintain a healthy, viable population.
ACKNOWLEDGEMENTS
We thank Carlos Solano, Ramsa Chaves-Ulloa, Zak Gezon and the staff at Cuerici Biological Station for their advice and assistance.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
ABIOTIC AND BIOTIC FACTORS AFFECTING THE GROWTH OF PALMA MORADA 
*(Prestoea acuminata)* IN A REGENERATION PROJECT IN Cuericí, COSTA RICA

GILLIAN A. O. BRITTON, EMILIA H. HULL, ELIZA W. HUNTINGTON, AND KALI M. PRUSS
Faculty Editor: Matt Ayres

Abstract: Anthropogenic activity, such as urban development and agricultural land-use, has led to frequent extirpation of local flora and fauna. Projects to regenerate and reintroduce such populations can be a tool in restoration ecology. Understanding the biotic and abiotic factors necessary for species survival is vital to ensuring a successful re-growth of the population. In Cuericí, Costa Rica, *Prestoea acuminata* (Spanish common name: Palma morada) was nearly eliminated from the Quebrada los Leones canyon in which they were once abundant. A project to regenerate Palma morada began in 2010. Four months after the transplantation of the palms to the canyon, we measured their growth with respect to local environmental conditions. Elevation, soil, temperature and water availability were all related to the growth of *P. acuminata*, while light availability was not. Plants higher up in the canyon, with greater access to water and a cooler environment were generally the tallest plants and had the highest survival rate. This project is an example of restoration ecology enacted at a local scale.

Key words: conservation, *Prestoea acuminata*, restoration ecology

INTRODUCTION
Anthropogenic activity, such as urban development and agricultural land-use, has led to the extinction of many of the world’s flora and fauna. Thus, reviving declining species is relevant to restoring ecosystems and maintaining biodiversity (Thomas et al. 2004). The loss of a single species can have cascading effects that fundamentally change the nature of the ecosystem (Ramirez et al. 2011). In an attempt to curb the destruction of ecosystems, conservationists and restoration ecologists are trying to regenerate and reintroduce endangered or extinct populations.

The challenges faced in restoration ecology (Allen et al. 1997) frequently include limited understanding of the ecological factors that influence growth and survival of the focal species. Our study focused on understanding the abiotic and biotic factors that influence the growth and survival of *Prestoea acuminata* (Spanish common name: Palma morada). In Cuericí, Costa Rica, Palma morada was apparently reduced to only a single palm remaining in a forest where it was once abundant (Carlos Solano, personal communication).

Costa Ricans have traditionally harvested Palma morada for the ‘heart of the palm’; the edible apical meristem (Haber et al. 2000). To harvest the heart of palm, the entire plant is killed. As the plant takes around 80 years to reach maturity (Zuchowski 2005), many people also harvest the juvenile Palma morada for its tender trunk. Cutting the trunk not only kills the individual, but introduces a fungus that spreads to surrounding palms (Selano 2013). Palma morada was one of only three species of palms in the forests of Cuericí and thus added functional diversity to the ecosystem. Palma morada, like many palm species, serves as a food source for birds including quetzals and parrots (Zuchowski 2005), contributing to the structural complexity of the understory and subcanopy layers of primary and secondary tropical forests (Haber et al. 2000).

To combat the loss of the Palma morada, Carlos Solano, the owner of Cuericí Biological Research Station and trout farm, transplanted approximately three hundred greenhouse-grown saplings (derived from the local seed source) into two small canyons in the montane forest. Environmental heterogeneity in abiotic factors, such as elevation, temperature, canopy cover, nutrients, and water availability, have strong general effects on plant growth (Svenning
In this study, we aimed to characterize the environmental features that influence site suitability for this species. Palma morada is found at high elevations in cloud forests (Zuchowski 2005) and has been reported to be a dominant species in older secondary forests due to its slow, steady growth (Marín-Spiotta et al. 2007). This implies that transplanted Palma morada would grow best at higher elevations, lower temperatures and with increased access to water and sunlight. We compared growth and survival of the transplanted palms across the range of environments in which it had been transplanted, and characteristic attributes of the microsites that were hypothesized to be of ecological importance. We also marked all mapped plants, and archived our plant-specific measurements, to facilitate continuing studies of the newly established palm population.

METHODS
Study system
All Palma morada seeds were obtained from a single adult palm at a neighboring farm. The palms were planted and cared for in individual pots in a greenhouse until being transplanted in October 2012 to four locations: the upper part of the Quebrada los Leones canyon (Lion Creek), the lower part of the canyon, a section of a neighboring canyon, and a garden plot.

In the upper canyon the palms were mostly planted in pairs, approximately 5-20 m apart, with a total of 195 palms in 103 sites along a 500 m stretch (Appendix Map 1). We identified three different habitat types: aboveground stream (stream), landslide zone (landslide), and a streambed that was dry at the time of measurement (no surface water flow).

In the lower canyons, the palms were almost all planted alone, with a total of 54 palms in 53 sites over a 250 m stretch (Appendix Map 2). We did not perceive environmental strata or gradients within this stretch of the canyon, and thus analyzed the lower canyon as one group. In the farm, there were another 380 palms still in individual containers, arranged in rows in a two by five meter plot. These palms were six months older than the palms planted in the field.

Procedure
We conducted this study on 29-31 January 2013 at Cuericí Reserve, Costa Rica. At each site, we recorded geographic coordinates and elevation using a Garmin GPSmap 76CSx to construct a map of the upper and lower canyons. For each plant we recorded the height of the entire plant, the stem, and the shoot; the length of the longest leaf; and the number of green and brown leaves. We also counted the number of leaflets that had been damaged by herbivores and scored each as 1-25%, 26-50%, 51-75% or 76-100% herbivory damage.

To evaluate potentially important abiotic factors for Palma morada, we measured light availability, temperature, and moisture between environments in the different parts of the upper canyon. To analyze light availability, we took a photo pointed directly up above each site using a Canon Digital Rebel XT and used ImageJ to estimate the percentage canopy cover. We used HOBO TidbiT waterproof temperature loggers to record shaded air temperature every 30 minutes at four sites for 36 hours: the greenhouse, the palms in the farm, the top of the upper canyon, and at the bottom of the upper canyon. Due to limited numbers of probes, we were unable to place temperature probes in either of the lower canyons. At one site in each of the three main environments in the upper canyon, we extracted soil cores (2cm diameter x 30cm depth) and examined them for visible layers and qualitative moisture content.

Statistical Analysis
To test the difference in success of the palms between the upper and lower canyons, we
compared plant height, which seemed the best proxy of overall plant success, with a paired t-test. We compared survivorship between the two canyons using a Fisher’s exact test. We also tested for variation in plant growth among the three environment types in the upper canyon; for this we used a nested ANOVA with site as a random effect nested within environments.

To examine the relationship between elevation and plant growth in the upper canyon, we ordered the plants by elevation, then grouped them into four elevation categories. We excluded the lower canyon from our elevation analysis because the lower canyon was spatially distant from the upper canyon. Moisture levels, wind, and humidity all seemed to differ greatly between the two canyons, which we assumed would be a bigger driver in plant growth than elevation. We used ANOVA to test for growth difference between habitat types.

To test whether plant success varied with light, we regressed plant height versus log transformed canopy openness. For all analyses we used JMP 10.0 and verified that all statistical assumptions were satisfied.

**RESULTS**

The palms in the upper canyon were about 117% taller than those in the lower canyon (t=4.22, df= 65, P < 0.0001, Figure 1). Palms in the upper canyon also had higher survival than in the lower canyon (1 out of 193 dead versus 4 out of 49 dead).

In the upper canyon, palms growing near the stream grew ~117% more than those in the dry streambed or landslide area (F2,114 = 6.61, P = 0.0019, Figure 1). Soil near the stream was relatively moist, dense, and contained many plant roots. The dry streambed, which dominated the canyon, had some moisture in the O-layer and a denser, moist layer approximately ten centimeters below the surface. Soil in the landslide area was dry and rocky with no noticeable organic layer. Plant height in the upper canyon also varied with elevation (F3,190 = 3.41, P = 0.019, Figure 2), with the upper plants being taller than those in the bottom half of the canyon, though we cannot determine at which elevation category the plant height differed significantly. Air temperature was consistently about 1.02 °C higher at the bottom of the canyon than at the top (Figure 3). Canopy cover (± SD) averaged 24.87± 15.83% (range = 4.56-75.91%). There was no correlation between canopy cover and plant height.

The nested ANOVA of plant heights in the upper canyon also revealed significant variation among sites within strata (estimated SD = 4.65cm)

**DISCUSSION**
The growth of Palma morada varied across environments: palms planted at the top of the Quebrada los Leones canyon were the tallest plants, while those in the lower canyons were the shortest. Additionally, survival was much higher in the upper canyon than in the lower canyon. Height varied with elevation, water availability, and temperature, but not with light. Apparently light was not limiting to Palma morada plant growth across the range of variation where I had been planted. Palma morada are reported to be high altitude palms (Zuchowski 2005), which is consistent with our result of higher growth at the top of the upper canyon (Quebrada los Leones).

Plants in the stream grew 138% more than plants in the lower canyons, a remarkable difference for such slow-growing palms. As the seeds all came from one tree, this is presumably all due to local environmental differences. Further studies could investigate whether planting palms as individuals is more or less advantageous than planting in pairs or in grouped plots.

Palma morada are extremely slow growing plants, taking nearly eighty years to reach maturity. The height variation among plants in the field (range= 21-64.5 cm) will likely become amplified as the plants continue to grow. It remains unknown how annual fluctuations of water availability and temperature will affect further growth. Finally, as the plants become larger it will also become

---

Figure 2. Plants in the highest part (mid-high: 48.6±1.165, high: 48.5±1.17) of the upper canyon grew more than plants in the lower half of the canyon (mid-low: 44.7±1.15, low: 45.01±1.15)

Figure 3. Temperature at the bottom of the canyon was consistently higher than at the top of the canyon (x-bar = 1.02 degrees C). We omitted some data due to differential sunlight exposure between the two temperature probes.
possible to assess effects of this previously abundant species on the rest of the biological community.

Thus far, the regeneration project has been very successful - all but 5 plants have survived and most appear to be healthy and producing new leaves. While it remains unclear how these palms will fare in the coming decades, the project is an example of how restoration efforts can be enacted at a local scale. Local landowners and farmers may frequently have knowledge of local ecology that can benefit reintroduction programs.

Furthermore, regeneration projects are often long-term, as growth takes time. Support from landowners and citizens is thus essential in both the implementation and maintenance of restoration projects. As human activity leads to increasing numbers of species becoming endangered, restoration projects will grow in importance.

ACKNOWLEDGEMENTS
We thank the staff of the Cuercí Biological Research Station for providing sustenance, Don Carlos for his inspiration, expertise, help and time, and Zak Gezon and Ramsa Chaves-Ulloa for their support.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED


Selano, C. 2013. Personal communication.


Proyecto de regeneración/ Regeneration Project
Mapa de las Palmas meroidas (Pristilophus occuminitus) transplantadas
Map of transplanted Palmis merolas (Pristilophus occuminitus)
Mapa de la Estación Biológica
Map of the Biological Station
Cuerici, Costa Rica
DAVID TAKES DOWN GOLIATH: INTERACTIONS BETWEEN *ECITON BURCHELLI*, *ECITON HAMATUM* AND *NASUTITERMES EPHRATAE*

SETH A. BROWN, JIMENA DIAZ, ELIZA F. HUNTINGTON, AND KALI M. PRUSS

Faculty Editor: Matthew P. Ayres

Abstract: Interactions between predator and prey can have strong consequences, for both populations often resulting in high selection pressure and rapid adaptation. According to the life-dinner principle, generalist predators exert strong selective pressure on their prey, allowing the prey species to evolve defenses to escape the predator. An interesting example of this phenomenon is the relationship between army ants, a voracious generalist predator, and termites, an herbivorous eusocial insect. A previous study reported that army ants actually avoided termites, but the mechanisms and causes were unclear. To further elucidate this relationship, we investigated termite defense against two species of army ant, *Eciton burchelli* and *Eciton hamatum*. Soldier termites actively defend their colony and have both mechanical and chemical defenses. We found that termite soldiers displayed unreciprocated aggressive behavior towards both species of army ant. Interestingly, our results suggest that army ant avoidance of termites is a response to a chemical cue specific to all castes of *N. ephratae* and not a response to chemical defenses specific to termite soldiers. Our findings indicate that some seemingly of potential prey items escape predators through innate intimidation.

Keywords: army ant, *Eciton burchelli*, *Eciton hamatum*, life-dinner principle, *Nasutitermes ephratae*, termite

INTRODUCTION

Interactions between predator and prey constitute some of the most conspicuous and interesting relationships in nature. Predator-prey interactions typically exert selection pressure on both species involved (Taylor 1984). An adaptation in one lineage, (predator or prey) can give rise to counter-adaptations in the other, eventually escalating into an evolutionary arms race (Dawkins and Krebs 1979). Various factors may grant one side an advantage in such an arms race. For example, according to the life-dinner principle (Dawkins and Krebs 1979), selection pressure is greater on animals fighting for their lives than on those that are only looking for their next meal. Thus, for generalist predators, whose interaction with a prey species is not necessary for survival, unequal selection pressure can allow prey species to evolve faster than the predator.

Army ants are voracious predators that live in colonies of up to one million, feeding on a wide variety of invertebrates (Franks and Holldobler 1987). Army ants are highly successful predators, often taking down prey many times larger than the individual ants (Franks and Holldobler 1987). Among the most abundant insects co-occurring with army ants are arboreal termites. Termites are eusocial insects that would presumably be an easy target for army ant as they live in large, immobile nests. However, previous observations have shown that army ants do not attack termite nests, but instead actively avoid encounters with termites (Carter et al. 2012). Surprisingly little literature addresses the nature of interactions between army ants and termites.

We investigated how a neotropical termite species, *Nasutitermes ephratae*, defends itself against two species of co-occurring diurnal army ant species: *Eciton burchelli* and *Eciton hamatum*. *E. hamatum* is an arboreal species of army ant, while *E. burchelli* is terrestrial. Therefore, it is likely that *E. hamatum* would have encountered *N. ephratae* more often than *E. burchelli* and thus should have a more highly evolved avoidance of termites. While worker termites have some defensive capabilities (Prestwich 1984), soldier termites’ specific job is to defend their colony. Soldiers have
two main mechanisms of defense: applying a poison with an extended labrum (similar to an upper lip), or ejecting a viscous, sticky solution, which irritates and mechanically immobilizes small attackers (Prestwich 1979). Because soldier termites have stronger defenses than the worker termites, it follows that army ants would have a stronger evasive response towards soldier termites than workers.

METHODS
We conducted our study from February 6-8, 2013, in Corcovado National Park, Costa Rica. We used five army ant colonies: three *E. burchelli* and two *E. hamatum* (species identity determined following Longino 2005). To understand potential differences in ant response to termite chemical cues versus live termites, we conducted trials with crushed termites (“chemical” trials), and observational trials of interactions between live organisms. We used crushed termites as a proxy for a termite chemical cue. We repeated all tests with both soldier and worker termites gathered from a nearby colony to determine whether there was a differential response to the two castes.

We performed the chemical trials on army ant trails that crossed tree roots. For each trial, we dragged a clean Q-tip (control) perpendicularly across the highway. We then haphazardly selected a treatment, swabbing in the same location with either crushed worker or soldier termites. At each colony, we performed the crushed termite trials on 12-16 root sections. We videotaped both control and treatment trials for 30 seconds post-swabbing. We later viewed the videos, analyzing response for 20 seconds. We categorized the behavior of every ant as either “no response” (no change in speed or direction when approaching the swab line) or “evasive response” (pause or change of direction).

To observe ant response to live termites, we performed two types of manipulation. At each ant colony we performed six trials where we placed three termites of one caste in the middle of the army ant highway and observed ant responses. To force termites and ants into direct contact, at each colony we placed three army ants in an enclosure (a small tupperware container) with 12 live termites. To determine how soldier and worker termites behaved towards ants when introduced simultaneously, we conducted some enclosure trials with a mix of both soldier and worker termites. We later conducted trials with only worker or soldier termites to distinguish between ant-termite interactions of each termite caste. We also observed and noted the outcome of all natural interactions between ants and termites that we happened upon during the course of our study.

Statistical Analysis
To test for differences in ant response between chemical treatments, we used ANOVA with a post-hoc Tukey HSD. We log10-transformed ant response to achieve normality. We used JMP 10.0 software for analyses and all assumptions were met for the analyses.

RESULTS
Both *E. burchelli* and *E. hamatum* displayed a strong response to crushed termites. Evasive behavior in response to termites was significantly greater than to a control ($F_{2,51} = 92.08, P < 0.0001$, Figure 1). Evasive behavior was not different between termite castes. There was no difference in response to crushed termites between the two army ant species.

Introduction of live worker and soldier termites into *E. burchelli* and *E. hamatum* highways resulted in obvious avoidance behavior by the army ants. In addition to individual evasive responses, we observed
instances of the focal colony diverting their entire highway around the introduced termites, and in a few cases ants assumed a seemingly aggressive posture (stopped, lowered the stinger, raised antennae). We observed both ant species performing this display in response to worker and soldier termites.

In arena trials between ants and termites, termite soldiers initiated aggression towards ants of either species; termite soldiers were observed spraying and climbing onto the ants. In one case, all twelve soldier termites were attached to one worker ant, immobilizing and eventually killing the ant. Interestingly, we did not observe the ants’ aggressive posture in arena trials. Ants never initiated aggressive interactions with the termites, but would respond aggressively if directly attacked. Interactions between termite workers and ants were almost never aggressive.

DISCUSSION
We found striking avoidance behavior from the army ants in response to termites. However chemical trials and live interactions elicited different behaviors in ants. Ants consistently avoided crushed termites, while some ants would remove termites from their path. Thus, it appears that ants display evasive behavior when only chemical cues are present, while they actively respond to the presence of a live termite. Moreover, ants sometimes exhibited a seemingly aggressive stinging posture when they encountered live termites. We speculate that this posture is a mechanism of communication to warn other ants that an enemy, rather than a prey item, is nearby.

We found no difference in army ant response to crushed soldiers versus workers, suggesting that both castes of N. ephratae contain the same chemical cue that ants respond to. Similarly, army ants in our enclosure trials avoided termites of both termite castes, even though termite workers never displayed aggressive behavior towards the ants. Hypothetically, if army ants initiated an attack on a termite nest, ants would have some amount of interaction with worker termites (though more with soldiers). Worker termites can also defend themselves (Eisner et al. 1976); thus, army ants would logically adapt to avoid both workers and soldier termites.

Army ants may be adapted to innately avoid termites. E. burchelli and E. hamatum, being generalist predators, do not need to attack heavily-defended termite nests when other more easily preyed-upon species are available. Termites successfully evade army ants because they have evolved defenses against a variety of predators, whereas ants can switch to a different prey item. This also explains the similarity between terrestrial and arboreal ant species’ response to termites; regardless of the frequency of encounters with termites, both are generalist predators.
predators. Even though the army ants are capable of destroying _N. ephratae_ nests, the costs of fighting termites apparently outweigh the benefits of the meal.

Surprisingly, our study found that the ant-deterrent mechanism is not soldier-specific. Future studies could investigate the evolutionary history between termites and army ants—did _E. burchelli_ and _E. hamatum_ adapt to avoid _N. ephratae_ specifically? Or is the avoidance behavior a relic of an evolutionary battle between ancestral species? Army ants’ avoidance of termites sheds light on asymmetrical predator-prey relationships and the ability of potential prey items to escape seemingly insurmountable predators. Such adaptations, which release prey from predation pressure, probably underlie the stable coexistence in many predator-prey systems.

**ACKNOWLEDGEMENTS**

We would like to thank the staff of Corcovado National Park for their accommodation and guidance, as well as Ramsa Chavez-Ulloa, Zak Gezon, and Matt Ayres for their helpful feedback.

**AUTHOR CONTRIBUTIONS**

All authors contributed equally.

**LITERATURE CITED**


COMMENSALISM AND TIDAL FORAGING IN ESTUARY BIRDS OF CORCOVADO NATIONAL PARK

AMELIA F. ANTRIM AND SAMANTHA C. DOWDELL

Faculty Editor: Matthew Ayres

Abstract: Optimal foraging theory posits that organisms will maximize energy gained and minimize energy spent while foraging, and will thus forage at times when food is most readily available. Estuary birds forage in an environment continuously fluctuating in abiotic factors and species composition, as marine fish and predators enter the system at high tide. We tested whether time of day or tidal cycling was a stronger driver of estuary bird foraging by observing bird abundance and species richness at two estuaries in Costa Rica, one with and one without bull sharks and large predatory fish at high tide. If tide is the major driver of birds’ foraging patterns, peak bird abundances and species richness should track tides and change between days as the time of high tide changes. If, however, circadian rhythms and solar time are the major drivers of bird foraging, we would expect to see no difference in time of peak abundance and species richness between observational days. We found that tidal fluctuation, not solar time, was a better predictor of bird abundance. Our data suggest a commensalism between large marine predators and piscivorous birds in which marine predators drive smaller fish toward the edges of the estuary. This relationship may increase bird abundance and species richness at high tide in Rio Sirena estuary. Flexible foraging patterns such as those exhibited in estuary birds may enhance understanding of optimal foraging theory in dynamic habitats.

Key words: commensalism, estuary, foraging, tide

INTRODUCTION

Optimal foraging theory predicts that organisms will forage in a way that maximizes benefits while minimizing foraging time and energy expenditure (Pyke 1984). Because food availability may fluctuate based on time of day and environmental conditions, organisms can minimize energetic costs by foraging when food is most abundant. Variable food availability and foraging patterns may be found in environments with daily dramatic fluctuations in abiotic conditions such as temperature, rainfall, wind, and tide (Menge 1972).

As dynamic ecosystems located at the interface of a freshwater river and the ocean, the abiotic conditions and species composition of estuaries fluctuate daily. Because of the unique nature of these systems, daily fluctuations in salt concentrations, water levels, and biotic community composition are affected by both the sun and the tide level (Allen et al. 2006). The influx of marine species during high tide provides enhanced opportunity for interspecies interactions, which may further influence optimal foraging by influencing the feeding behaviors via competition or beneficial relationships such as mutualisms and commensalisms.

The diverse and dynamic habitat that estuaries provide also requires inhabitants to compete with marine, shore, and river organisms. If tide level influences birds’ ability to obtain prey, the optimal time for estuary shore birds to forage should fluctuate on a daily basis with changing times of high tide rather than time of day alone. Deffenbach et al. (2012) examined the relationship between tide and the foraging patterns of Egretta thula (Snowy Egret), and hypothesized that the temporal correlation resulted from a commensalism with large marine predators that force smaller fish toward shore, where the fish are more accessible to the piscivorous birds.
We studied time patterns of foraging in the full bird community of the estuary. If patterns of bird presence track solar rather than tidal fluctuations, peak bird abundance and species richness should occur at the same time on both observational days. Conversely, if birds track tidal fluctuations, peak abundance and richness should change with the change in time of high tide.

We further tested the commensalism hypothesis by comparing the Rio Sirena estuary with the Rio Claro estuary, which contains relatively few large marine predators (Deffebach et al. 2012). Under the tidal commensalism hypothesis, the effect of high tide on bird presence should be amplified in the Rio Sirena estuary, where such predators are more abundant.

METHODS

Study Sites

The Rio Sirena and Rio Claro estuaries are located approximately 1500 m apart near the Sirena Ranger Station, Corcovado National Park, Osa Peninsula, Costa Rica. Both are approximately 50 m wide. Observational areas were approximately 75 m in length, measured downriver from the mouth of the estuary. The estuaries appeared to offer similar bird habitats. Crocodiles were noted in both lagoons, which differed from the 2012 study when they were not seen in Rio Claro. However, the presence of bull sharks and large predatory fish has been noted only in the Rio Sirena estuary.

Observational Methods

We conducted our study on February 7 and 8, 2013. Sites were observed in morning and afternoon shifts, alternating between the two sites (Table 1). Schedule was determined based on access to estuaries. Our observational periods consisted of 10-minute continuous trials throughout which we recorded all bird presences at the estuary in order to determine both abundance and species richness. Crocodile presences were noted. Shark presence could not be determined from the observational points at each estuary. High tide occurred at 12:06 pm on February 7 and at 1:09 pm on February 8.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Rio Sirena</th>
<th>Rio Claro</th>
</tr>
</thead>
<tbody>
<tr>
<td>07-Feb AM</td>
<td>8:00-8:50</td>
<td>9:30-10:20</td>
<td></td>
</tr>
<tr>
<td>07-Feb PM</td>
<td>13:10-14:10</td>
<td>15:15-16:15</td>
<td></td>
</tr>
<tr>
<td>08-Feb AM</td>
<td>9:50-10:40</td>
<td>8:05-8:55</td>
<td></td>
</tr>
<tr>
<td>08-Feb PM</td>
<td>15:30-16:20</td>
<td>13:55-14:45</td>
<td></td>
</tr>
</tbody>
</table>

Statistical Analysis

To visualize the raw data, we used R statistical software (R Core Team 2012) to plot bird abundance by minutes from solar noon and time from high tide for days one and two.

To fit a curve to bird abundance with relationship to tide, we used a sinusoidal function developed by Deffebach et al. (2012):

\[
Z = \left( \sin \left( \frac{\pi}{2} + (X - t - p) \cdot \frac{2\pi}{p} \right) + \left( n + \frac{a}{2} \right) \right)
\]

Equation 1. Developed by Deffebach et al. (2012).

We used maximum likelihood estimation to fit the sinusoidal curve to the data and estimate our parameters, constraining the minimum number of birds at the estuary as greater than or equal to 0 (JMP 10.0 software).

RESULTS

The bird species recorded during observations (in order of abundance) were: Semipalmated Plover, Whimbrel, Spotted Sandpiper, Least Sandpiper, Snowy Egret, Little Blue Heron, Mangrove Black Hawk, Tricoored Heron, Osprey, Flycatcher (only one morphospecies observed, could not be identified to species), Black Vulture, Tiger Heron,
Scarlet Tanager, Great Blue Heron, Willet, Blue-Winged Teal, Belted Kingfisher, Ringed Kingfisher, Chestnut-Mandibled Toucan, Franklin’s Gull, Yellow-Headed Caracara, Green Striated Heron, Morning Dove, and Dusky Antbird.

At Rio Sirena, bird abundance was estimated to be highest 3 minutes before high tide (SE = 15, Figure 1a). At Rio Claro, bird abundance was estimated to be highest 55 minutes after high tide (SE = 49, figure 1b). The mean difference between the highest and lowest estimated bird abundances was 44 birds at Rio Sirena and 3 birds at Rio Claro (Table 2).

Table 2. Parameter estimates ± SE for Equation 1 for peak bird abundances at Rio Sirena and Rio Claro Estuaries. Parameter \( t \) represents time relative to high tide at which abundance peaked (minutes), \( p \) represents the period of the tidal cycle (fixed at 737 minutes), \( n \) represents the minimum number of birds present (nadir), and \( a \) describes amplitude (difference between highest and lowest abundances).

<table>
<thead>
<tr>
<th>Param eter</th>
<th>Mean Rio Sirena</th>
<th>Standard Error Rio Sirena</th>
<th>Mean Rio Claro</th>
<th>Standard Error Rio Claro</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t )</td>
<td>-3</td>
<td>15</td>
<td>55</td>
<td>49</td>
</tr>
<tr>
<td>( p )</td>
<td>737</td>
<td>N/A</td>
<td>737</td>
<td>N/A</td>
</tr>
<tr>
<td>( a )</td>
<td>44</td>
<td>14</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>( n )</td>
<td>0</td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Species richness was estimated to be highest at Rio Sirena 12 minutes after high tide with a standard error of 18 minutes (Figure 2a). At Rio Claro, species richness was estimated to be highest 42 minutes after high tide with a standard error of 23 minutes (Figure 2b). The mean difference between the highest and lowest estimated species richness was 7 species at Rio Sirena and 4 species at Rio Claro (Table 3).

Table 3. Parameter estimates for Equation 1 for peak species richness at both Rio Sirena and Rio Claro. See parameter definitions in Table 2.
We plotted raw bird abundance data as a function of time of day (Figure 3a) and time relative to high tide (Figure 3b), revealing a smaller distance between peak abundances when explained by time relative to tide.

**DISCUSSION**

Bird abundance and species richness covaried more closely with tide than with time of day (Fig. 3), suggesting that birds can forage more efficiently at estuaries when tide levels are high. Tidal effects on foraging could be related to either of two factors. First, birds may be more able to acquire more prey due to increased prey concentration following the influx of marine fish at high tide. Alternatively, birds may be able to acquire prey more efficiently at high tide as a result of a commensalistic relationship with marine predators in which small fish are forced to the shores of the estuary (Deffebach et al. 2012).

While both Rio Sirena and Rio Claro estuaries experienced an increase in bird abundance and richness at high tide, tidal effect on foraging patterns was stronger in the Rio Sirena estuary than in the Rio Claro estuary. At Rio Sirena estuary, bird abundance and species richness peaked within a few minutes of high tide. While Rio Claro estuary experienced peaks in bird abundance and species richness shortly after high tide, the relationship between tidal cycle and abundance and species richness was not as strong. The weaker relationship with tidal cycles and the decreased difference between lowest and highest bird abundance at Rio Claro estuary suggest that tide does not affect bird foraging as strongly as in Rio Sirena.

Because Rio Sirena contains more large marine predators than Rio Claro, the stronger tidal effect at Rio Sirena suggests a commensalism between large marine predators and piscivorous birds. Small fish may be more accessible to piscivorous birds at high tide due to an influx of large marine fish and sharks, which force the fish to the sides of the estuary, as suggested by Deffebach et al. (2012). While Deffebach et al. (2012) noted that large marine predators were absent from the Rio Claro estuary, we observed two crocodiles in the estuary on each visit. Therefore, tidal effects on this commensalism would be driven specifically by the influx of marine predators such as large predatory fish and bull sharks, as crocodiles can be a constant presence in both estuaries regardless of tide level.
Alternatively, fish may be more abundant throughout the estuary at high tide due to an influx of marine fish with the advancing tide. If the tidal effect on bird foraging is due to increased fish abundance rather than a commensalism with marine predators, then the decreased effect of tide at Rio Claro estuary would imply that fewer marine prey fish enter this estuary than the Rio Sirena at high tide.

At Rio Claro, bird abundance and species richness peaked not at high tide, but 42 and 54 minutes after high tide, respectively. One potential explanation for this pattern is an increased exposure of foraging surfaces shortly following high tide. At Rio Claro, birds often foraged on rocky patches that were submerged at high tide. Species richness and abundance may have peaked shortly after high tide at Rio Claro because marine fish and other potential prey items enter the estuary at high tide, yet optimal feeding time occurs only after this desirable feeding patch becomes exposed when the tide recedes, uncovering organisms on and around the exposed area.

In addition to tidal level, foraging efficiency may be affected by interspecific interactions among birds. While most estuary birds were solitary or traveled in monospecific groups, we frequently observed mixed flocks of whimbrels and semipalmated plovers at Rio Sirena estuary. As competitors for nematode and macro invertebrate prey, these two species would not be expected to forage together unless mixed flocking behaviors benefit the birds in some way. One possible explanation for this behavior is increased vigilance in predator detection. Alternatively, the birds’ differing beak morphologies suggest that they may capture prey from different depths in the substrate; plovers and whimbrels may feed in a processing chain commensalism, in which one species uproots or prepares food for the other.
Our results suggest that birds adjust their foraging patterns based on tide level at the two estuaries, thus altering their daily patterns of behavior as the time of high tide changes. However, the optimal foraging period with respect to tide may vary between estuaries due to factors such as interspecies relationships and foraging substrate. In such a dynamic ecosystem as an estuary, plastic daily foraging patterns could greatly increase foraging success. Understanding the variability of foraging patterns in response to fluctuating environmental variables may help inform our understanding of optimal foraging theory in dynamic environments.

ACKNOWLEDGEMENTS
We thank Matt Ayres for his assistance in data analysis and use of his binoculars. We thank Elise Seyferth for her help with bird identification, and Zak Gezon for his help with statistical analysis and software.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
COSTLY SIGNALS: MEASURING THE COST OF DEWLAP DISPLAY BY NOROPS LIZARDS

GILLIAN A. O. BRITTON, MARIA ISABEL REGINA D. FRANCISCO, AND ELISABETH R. SEYFERTH

Faculty editor: Matthew Ayres

Abstract: Intraspecific interactions often involve communication between individuals through various signals. In many cases, sexual selection drives the evolution of dramatic physical displays. Evolution of dramatic displays requires that the benefits of the signal outweigh the costs of producing and using the signal. However, many displays seem energetically costly to produce and appear to limit movement while increasing predation risk. We investigated whether dewlap displays of Norops spp. (anoles) are energetically costly signals by measuring how dewlap color and size relate to anole running speed (a proxy for predator evasion) and body size (important for mating success and territorial defense). Variation in speed was not related to dewlap size, suggesting that dewlap size is not a signal for or a cost to speed. There was also no relationship between dewlap color and either speed or body size. However, larger dewlaps tended to be on larger anoles, implying that dewlap size is an honest signal for body size and does not constrain growth. We also investigated how dewlap size and body size influence dewlap displays by males defending a territory. We found no influence of dewlap size on dewlap displays; however, defender anoles were less likely to use dewlap displays when their body size differed greatly from that of invader anoles. Dewlap displays might minimize escalation of conflict by signaling body size. Sexually selected traits are not necessarily costly. The potential for low costs and high benefits may help explain the high frequency and striking variety of sexually selected traits in nature.

Key words: dewlap, Norops spp., sexual selection, signaling theory, territoriality

INTRODUCTION

Intraspecific communication often relies on dramatic physical displays thought to evolve in response to sexual selection (Hoefler et al. 2008). For such dramatic displays to evolve, it is expected that the benefit of the signal outweighs the costs of producing and using the signal. However, phenotypes such as the antlers of a moose, the bright inflated throat of a frigatebird, and the long tail of a quetzal often seem energetically costly to produce and appear to limit movement and increase predation risk. Many displays, such as moose antlers, are used in both attracting a mate and in competing with other males. While the costs of displays are well-known, we seek to weigh the costs of sexually selected traits against the benefits of decreased aggressive interactions and greater mating success.

Norops spp. (hereafter referred to as anoles) frequently use dewlap displays in male-male territorial interactions and in courtship of females. Anole dewlaps are brightly colored and make the otherwise cryptic lizards visible to birds, their main visual predators (Wundele 1981). However, male-male formalized displays also enable individuals to assess their opponents and thus avoid fights they are unlikely to win (Payne and Pagel 1997), suggesting that dewlaps may also reduce cost to male anoles. Therefore, we questioned whether dewlap size and brightness are costly to anoles in terms of running speed and body size. If relatively large dewlaps reduce running speed, anoles may have to balance a tradeoff between evading predators and mating success (as females prefer larger and brighter dewlaps; Sigmund 1983). If anole males with larger and brighter dewlaps are also faster and larger, then dewlap size represents an honest signal (Vanhooydonck 2005). Alternatively dewlaps might be noticeably variable among males of the same size and could be inversely related to running speed, representing a dishonest signal.

The use of dewlaps as a signal may provide benefits that counterbalance potential costs of energy and exposure to predators. To determine the strength of the dewlap signal in male-male interactions, we tested how the relative size of the defending anole and an intruder affects dewlap use. Larger anole body size is linked with increased territorial ability and may explain the home advantage of a defending anole in male-male conflicts (Losos 1990); additionally larger anoles are more aggressive than smaller anoles (Takarz 1985). Therefore, larger anoles
may be more likely to use their dewlap display than smaller anoles regardless of the size of the intruding anole (and therefore expose themselves more to predators). Alternatively, if smaller anoles use their dewlaps more frequently it would suggest that dewlap display is used to compensate for smaller body size. If dewlaps are only used when anoles are close in size, it would imply that dewlaps are redundant signals and are used only when size differences are not sufficient to indicate the outcome of a conflict.

METHODS

Data Collection

We conducted all fieldwork at the Sirena Biological Station in Corcovado National Park, Puntarenas, Costa Rica on 7–8 February, 2013. To test how anole dewlap size and color affected running speed, we caught 20 male anoles from 0800-1100 and from 1330-1500 on the Espaveles trail on 7 February. We measured the snout-to-vent length (SVL) to characterize lizard body size, which can be regarded as a proxy for fitness (Blob 1998). We manually extended each anole’s dewlap and measured size of dewlap directly adjacent to the body along the anteroposterior axis (dewlap length). We also measured the size of the extended dewlap along the dorsoventral axis (dewlap extension). To quantify dewlap color, we took a picture of each extended dewlap. Using Microsoft Paint software, we isolated a 20 by 20 pixel square in the center of the dewlap and compressed it into 1 pixel. We recorded saturation and redness (R((R+G+B)/3)) of the pixel. To measure sprint speed, we constructed a cardboard tube (5 cm diameter and 30 cm in length) with a 2 cm wide clear panel of Saran wrap inserted down its length and with centimeters marked along its length. We placed each anole at the mouth of the tube and allowed it to run through while we filmed it with a Canon PowerShot S95 camera. We analyzed the video to calculate average sprint speed over a distance of 10 cm or longer using EagleEye Proviever 0.8.16 software by EagleEye Digital Video, LLC. We measured each individual 1-3 times and analyzed the highest sprint speed that we observed for each anole.

To test how dewlap size was related to body size and how dewlap displays were used in territory defense, we caught 16 male anoles on the Guanacaste and Rio Sirena Trails on 8 February from 0745-1100. For each anole (the “invader”), we recorded SVL, dewlap length, and dewlap extension. We then haphazardly selected another male anole (the “defender”) that was in residence on a nearby tree trunk and within 1 m of the ground. We held a cardboard tube (5 cm in diameter and 30 cm in length) at approximately 0.5 m above the ground and 0.75 m from the tree and pointed it directly at the defender’s tree. We placed the invader in the end of the tube furthest from the tree and released the anole. If needed, we gently prodded the invader down the tube until it dropped to the ground or jumped a short distance from the tube’s end. Upon leaving the tube, we observed both anoles for two minutes and recorded the following for the defender: number of times dewlap was opened and closed, the total length of each display (number of seconds dewlap was extended), number of head bobs (number of times anole moved head up and down while dewlap was extended), and number of push-ups (number of up-and-down body movements produced by bending the front legs) (Forster et al. 2005). When the trial ended, we captured the defender and measured SVL, dewlap length, and dewlap extension.

Statistical Analyses

All analyses were conducted using JMP 10.0 statistical software and the data met assumptions for all tests performed. We tested if dewlap size (regardless of body size) was a signal of speed by plotting a linear regression of speed vs. dewlap extension. We also tested how dewlap color was related to sprint speed and body size by plotting regressions of speed and size vs. redness. We further tested if dewlap size was an honest signal of body size by plotting a linear regression of body size vs. dewlap extension.

We tested how dewlap display was affected by the defender’s dewlap size by plotting regressions of each dewlap display metric vs. dewlap extension length. Then we tested how these dewlap display metrics were related to the size of the defender relative to the invader (defender SVL - invader SVL). We also tested
whether the probability that a defending anole would display at all was related to the size difference between defender and invader. For this, we performed a t-test of the difference between lizard sizes (defender SVL - invader SVL) of anole defenders that did and did not perform during the interaction (display or no display). We further tested whether the probability that a defending anole would display at all was related to a non-directional difference in size by running a t-test of the absolute difference between defender and invader size (absolute value(defender SVL - invader SVL)) of anole defenders that did and did not perform during the interaction.

RESULTS
There was no relationship between absolute dewlap size and sprint speed nor between dewlap redness and either sprint speed or SVL. However, SVL was greater in anoles with larger absolute dewlap size (Fig. 1; R² = 0.56, P < 0.0001). There was no relationship between dewlap size (adjusted for body size) and dewlap display duration, number of dewlap displays, number of head-bobs, or number of pushups. However, the use of a dewlap display was significantly more likely if the invading and defending anoles were similar in body size (Fig. 2; F₁,₁₂ = 3.28, P = 0.048) regardless of whether the defender was the smaller or larger anole. Dewlap displays were used when the absolute value of difference in SVL between defender and invader was 2.75 ± 1.42 mm (mean ± SE), while dewlap displays were not used when the SVL difference was 7.06 ± 1.91 mm (mean ± SE).

DISCUSSION
Larger dewlaps did not confer detectable physiological costs to anoles. Variation in speed was unrelated to dewlap size, suggesting that (1) there is no tradeoff between absolute dewlap size and speed and (2) dewlap size is not a signal of speed (an indicator for predator evasion). We also found no relationship between dewlap color and either speed or body size, although Steffen (2008) reported that bright coloration was related to body condition, providing a signal of anole robustness. Bright coloration may be correlated with traits that we were unable to measure, such as immune response, fecundity, or body condition.

Larger dewlaps were on larger anoles (Fig. 1), suggesting that dewlap size may be an honest signal for traits associated with body size, such as foraging success and territoriality, while not decreasing body growth (Losos 1990). However, if female anoles use dewlap size as a signal for mates with larger body size, selection would favor smaller anoles with larger dewlaps. The honest quality of dewlap size as a signal for body size might be maintained by situations in which both body size and the dewlap display function, such as in male-male territorial conflict.

Our study suggests that dewlap size is a redundant signal used when body size differences between anoles are not large enough to indicate the likely outcome of a male-male encounter. Males were more likely to use the dewlap signal when a similarly sized anole was introduced than when size differed greatly between the lizards (Fig. 2). When size difference is great, observing the size disparity alone may allow two anoles to determine the probable outcome of a fight without using the dewlap display and potentially revealing
themselves to predators. However, when anoles are close in size, they may prevent a potentially costly fight by also using dewlap signals. While dewlaps do not indicate anole speed, they do indicate size and apparently the fighting ability and territoriality of the defender.

Our results therefore suggest that the combination of rival size assessment and use of dewlap displays may help anoles to minimize costly physical interactions when defending territories. In fact, we observed only one defender-invader encounter escalate into physical biting out of seventeen total trials. Male animals such as red deer often perform formalized displays at a distance using signals such as antlers to decrease the escalation of conflict and reduce the incidence of physical attack (Payne and Pagel 1997). Likewise, many bird species and poison dart frogs (e.g., Dendrobates pumilio) use vocalizations to ward off intruders from their territory and thus reduce male-male conflict (Bunnell 1973, Peek 1972). Anole dewlap displays may play a similar role in reducing the escalation of male-male interactions to physical attack.

While further study is necessary to more thoroughly quantify the costs and benefits of dewlaps and dewlap displays, our results suggest that the cost of sexually selected traits may not be as great as they first appear. Dramatic displays may sometimes have limited or trivial costs in terms of energy or predator exposure while providing great benefits by reducing the occurrence of potentially damaging physical conflict. The selective use of displays based on situational context may further reduce costs. The apparently low cost and high benefit of some sexually selected traits helps explain the development of the great variety of sexually selected displays involved in intraspecies interactions.

ACKNOWLEDGEMENTS
We would like to thank the staff of Corcovado National Park for room and board and access to the park trails and Ramsa Chaves-Ulloa and Zachariah Gezon for their support.

AUTHOR CONTRIBUTION
All authors contributed equally.

LITERATURE CITED
Sigmund, W. R. 1983. Female preference for Anolis carolinensis males as a function of
dewlap color and background coloration. Society for the study of amphibians and reptiles. 17: 137-43
BIGGER IS BETTER BUT MORE DEMANDING: KLEPTOPARASITES, MALES, AND METABOLIC NEEDS OF *NEPHILA CLAVIPES*

ELLEN T. IRWIN, MOLLY R. PUGH, AND VICTORIA D. STEIN

Faculty Editor: Matt Ayres

Abstract: As animals grow larger, they face tradeoffs in terms of energy budget, foraging ability, and reproduction. The golden orb-weaving spider, *Nephila clavipes*, grows dramatically over its lifespan (carapace lengths of 3-21 mm in web-building individuals). The variation in body size may affect spiders’ reproductive opportunities, parasite load, and physical ability to capture and consume prey. Large spiders may be driven to attack prey indiscriminately because of their enhanced capture and consumption ability. Alternatively, their increased effectiveness at prey capture might allow larger spiders to be choosier in prey selection. To test these competing hypotheses, we placed large and small prey in variously-sized *N. clavipes* webs and measured the time to attack. We found that larger spiders consumed a wider variety of prey, likely to compensate for their higher metabolic costs and the greater number of males and kleptoparasites found in their webs. Larger predators must support their higher metabolisms and parasite loads with increased caloric intake, and accomplish this in part by exploiting a larger range of prey sizes.

Key words: *Nephila clavipes*, predator-prey size difference, parasitism

INTRODUCTION

As an animal grows larger, it needs to consume more to maintain its higher metabolism (Isaac 2005). On the other hand, it can also have greater capacity to capture and consume food items (Shine 1991; Isaac 2005). In the case of predators, larger animals are generally able to handle larger prey items, allowing them a wider range of potential food with less risk to themselves (Shine 1991). The ability to consume a wider range of foods can give a reproductive individual an advantage as well, providing it with more nutrients to dedicate toward producing offspring (Cohen et al. 1993; Higgins and Rankin 2001). Another cost of being large can be increased parasite load (Isaac 2005).

The golden orb-weaving spider, *Nephila clavipes*, is commonly found creating and occupying webs on human structures, along trails, across streams, and around forest clearings in woody areas of Central and North America (Robinson and Mirick 1971). It constructs one sticky fine-meshed orb web, usually U-shaped with the hub offset toward the top; the orb web is surrounded by a series of barrier webs situated at various angles, possibly intended as protection from predators. *N. clavipes’* typical prey include flies, bees, wasps, and small lepidopterans (Robinson and Mirick 1971).

*N. clavipes* grows significantly over its lifespan (Higgins and Rankin 2001). We observed carapace lengths ranging from 3.5 to 20.7 mm (none of which were newly-hatched spiders). Increased size may increase spiders’ physical ability to capture and consume prey. Smaller spiders are limited in the prey they can access based on two factors. First, they have more difficulty capturing large prey items. We observed that smaller spiders built smaller, more delicate webs while larger spiders built larger, studier webs of thicker silk. The quality of the tool (the web) that spiders use to capture prey improves as the spider grows. Second, large prey, which can be larger than the spider itself, pose more threat of injury or extreme energy expenditure to small spiders than large spiders (Henaut et al. 2001).

Along with their increased effectiveness at capturing prey, larger *N. clavipes* are also likely to have greater reproductive success. In the final instar, when females are reproductive, their fecundity increases with larger body size, allowing females to lay more eggs of higher quality (Higgins 1992). Males of a closely related species (*N. plumipes*) searching for mates choose females based mainly on size and evident fecundity (Kasumovic et al. 2006). Thus, male
N. clavipes may tend to be more numerous in the webs of larger females. Alternatively, they might be dispersal limited or might distribute themselves across webs (even those of smaller females) to minimize male-male competition.

In addition to male N. clavipes, other spider species can share the females’ webs and sometimes their prey. These kleptoparasites and kleptobionts build their own webs adjacent to or even on and within N. clavipes webs and either catch their own prey or steal directly off the females’ webs (Herberstein 2011). As with the male N. clavipes, it could be expected that larger N. clavipes would harbor more kleptoparasites and kleptobionts in their webs than smaller N. clavipes.

Larger N. clavipes have greater metabolic needs and greater capacity to capture and consume prey. They might attack a wider variety of prey, and attack faster than smaller spiders. Alternatively, larger spiders might be more discriminating in their prey choices because they have bigger webs and a larger spectrum of prey items that they could attack and consume.

METHODS

On 6-8 February, 2013, at Sirena Biological Station, Corcovado National Park, Puntarenas, Costa Rica, we located 36 female Nephila clavipes along the Guanacaste, Naranjo, and Espaveles trails. For each focal female, we measured the carapace length as an indicator of spider size and noted the number of males and cohabitants (kleptoparasites and kleptobionts) in its web. We then randomly assigned each spider to one of three prey treatments: cicada, leaf cutter ant, or stingless bee. We placed the previously-caught prey items in the web approximately 15 cm from the focal spider, and noted the time of attack measured from prey placement until the spider sank its fangs into the prey. We ended each trial after five minutes, regardless of whether the spider attacked the prey.

**Statistical Methods**

We tested for relationships between female spider carapace length and (1) number of males and (2) number of cohabitants in the web with linear regressions. We tested for a relationship between female spider carapace length and probability of attack with logistic regression. We
tested for effects of female carapace length on time to attack with a general linear model that included carapace length, prey type (ant, bee, cicada), and their interactions. We went on to compare the two prey of similar size (ant vs. bee) for differences in spider preference toward both the familiar and unfamiliar prey, and performed the same analysis for the two familiar prey of different sizes (bee vs. cicada). Data met the assumptions for all tests. All analyses were performed using JMP 10.0 statistical software.

Figure 2. The probability of attack, irrespective of prey type, increased logistically with spider carapace length. Predicted line represents best logistic fit on data.

Table 1. Percentage of each prey type that *N. clavipes* attacked.

<table>
<thead>
<tr>
<th>Prey type</th>
<th>% attacked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf cutter ant</td>
<td>50</td>
</tr>
<tr>
<td>Stingless bee</td>
<td>100</td>
</tr>
<tr>
<td>Cicada</td>
<td>60</td>
</tr>
</tbody>
</table>

**RESULTS**

The number of male *N. clavipes* in a female’s web increased with female carapace length (r² = 0.20, n = 36, P = 0.006; Figure 1A). The number of cohabitating spiders also increased with female carapace length (r² = 0.12, n = 36, P = 0.04; Figure 1B).

The probability of *N. clavipes* attack on prey increased logistically with female carapace length (chi-square = 4.85, P = 0.028, df = 1; Figure 2). Spiders attacked all bees, while they only attacked approximately half of all ants and cicadas (Table 1).

For the small prey items (ants and bees), time to attack was relatively rapid (ca. 35 s) and unrelated to spider carapace length (Figure 3). Large spiders attacked large prey (cicadas) as quickly as they attacked small prey, but the time to attack tended to be longer for medium size spiders, and small spiders did not attack cicadas at all (Figure 3).

**DISCUSSION**

The greater number of males in the larger females’ webs may be explained by male choice of larger, more fecund females, as well as female reproductive readiness—a female *N. clavipes* is not fertile until she reaches her final instar and her largest size (Higgins 1992). In addition, larger females with presumably better webs can probably nutritionally support more males (Kasumovic et al. 2007). The other cohabitants also occurred in greater numbers in the webs of the larger females, which is probably adaptive for the cohabitants because a larger web is likely to catch more prey and provide more structure for them to construct their own webs. *N. clavipes* are known to build semipermanent webs that they occasionally abandon in favor of building fresh ones; this behavior may have evolved to shed the accumulating load of parasites (Herberstein 2011). If so, larger *N. clavipes* should be more likely to change webs than smaller spiders with fewer cohabitants.

The community of *N. clavipes* males and other cohabitants on the larger females’ webs might contribute to the females’ higher attack probability on all prey items (Figure 1). Larger spiders must already have higher energetic needs due to their maintenance costs and reproductive investments. Smaller females, on the other hand, had fewer cohabitants to compete with, were
probably not reproductive, and therefore had more latitude to be discriminating in their prey choices, especially if the prey was large or likely to cause them harm, as in the case of cicadas.

*N. clavipes* are presumably unaccustomed to finding ants in their webs, because ants do not fly (other than alates, which are rare). Stingless bees must be common as prey. Perhaps due to this, spiders attacked all bees but only half of the ants placed in their webs (Table 1). Also, bees caused more vibrations in the web than the ants did, which may have elicited stronger responses by spiders. However, we found no difference in time it took for differently sized spiders to attack ants or bees (Figure 3). Both bees and ants are small prey, and even small spiders could, and did, attack them. Thus, while both large and small spiders had the same response time to small prey, spiders were overall less likely to attack the unfamiliar ants than bees.

Our study showed that larger female *N. clavipes* fed on a wider range of sizes of prey than did the smaller spiders. Of all cicada trials, the cicadas that were attacked (Table 1) were in larger spiders’ webs, while those that were not attacked were in smaller spiders’ webs. We observed that some small spider webs were too fine to capture cicadas at all. Small size apparently constraints spiders to small prey both because of their smaller webs and their reluctance to attack large items when they are trapped.

*N. clavipes* illustrates how larger organisms have a greater capacity to consume larger prey more efficiently. Larger spiders need to consume more to maintain their higher metabolism, but they also have greater capacity to consume larger items due to their stronger, larger webs. As a result, they have access to a wider range of prey sizes, which is vital to obtaining sufficient nutrients for reproduction, and can probably compensate for food taken by males and other cohabitants. *N. clavipes* exemplifies how organisms’ foraging abilities and behaviors change with size. All predators must support their higher metabolisms with increased caloric intake, sometimes accomplishing this by taking advantage of a larger range of prey sizes (Cohen et al. 1993, Shine 1991). When parasite load increases with size, these caloric requirements

---

**Figure 3. Time to attack cicadas, bees, and ants by female *N. clavipes* of variable size.**

![Figure 3](image_url)
become even more pressing and large predators are further challenged to meet their energetic needs.

ACKNOWLEDGEMENTS
We would like to thank Ramsa Chaves-Ulloa for her support in our project development, and the staff at Sirena Biological Station for accommodating our physical and scientific needs.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
HABITAT SELECTION IN EUGLOSSINE BEES IN CORCOVADO, COSTA RICA

TYLER E. BILLIPP, COLLEEN P. COWDERY, AND EMILIA H. HULL

Faculty Editor: Matt Ayres

Abstract: Habitat selection influences species distribution based on environmental factors and resource distribution. In plant-pollinator systems, the distribution of plants often affects the distribution and community structure of the pollinators. We examined this relationship in euglossine bees and orchids, which engage in an obligate mutualism. Orchids rely on euglossines for cross pollination, while euglossines collect scents from the orchids, presumably to increase their reproductive success. By deploying scent traps in primary and secondary forest light gaps and shade patches, we tested some of the factors potentially influencing euglossine habitat selection. We found a strong preference of bees for light gaps, measured in bee abundance. We also found that community assemblage, while of comparable evenness, differed between the two forest types. This finding is likely related to the distribution and types of orchids present at the tested sites, as well as the foraging ranges and routes of the euglossines. The differences in euglossine communities between primary and secondary forests probably reflect differences in habitat structures and resource availability.

Key words: community structure, euglossine, habitat selection

INTRODUCTION
Habitat selection is driven by multiple factors, foremost the need for suitable abiotic conditions and resources to survive and reproduce (Rosenzweig 1991). For example, male bowerbirds require both a physical bower for displays as well as a variety of bower decorations collected from their immediate environment to attract a mate (Borgia 1995). Habitat selection is also greatly affected by ecosystem heterogeneity; a fine-grained environment affords more opportunities for niche specialization as it harbors more microclimates, affecting resource distributions (Karr and Freemark 1983).

Among pollinators, habitat selection depends largely on the distribution of their plant resource (Sowig 1989). Pollinator distribution can be highly structured into sub-habitats, or cosmopolitan within an ecosystem according to the distribution of their plants. Among plant-pollinator associations, the euglossine bee-orchid relationship is highly derived. Euglossine bees and orchids occur in a co-obligate mutualism across the Neotropics (Ramírez et al. 2011). Only male euglossine bees pollinate orchids, and orchids attract male bees aromatic volatiles, not with food resources (Ackerman 1982). It is hypothesized that males collect the pungent scents as badges signifying their foraging achievements, which may be a proxy for fitness (Williams and Whitten 1983; Roubik and Hanson 2004). Orchids are generally epiphytic and tend to be more abundant in primary forest than secondary forest communities (Barthlott et al. 2001). However, since orchids tend to be heterogeneous on a fine scale (300-700 m), male euglossines must forage widely to locate orchid patches (Armbruster 1993). Once orchid “hot spots” are identified, males display high foraging fidelity toward them, flying from patch to patch (Janzen 1971).

A vital component of euglossine bees habitat selection must be based on orchid distribution; without orchids, euglossine populations crash (Ramírez et al. 2011). As choices involving habitat are crucial to the success of euglossine bees, we examined three influences of habitat preference, specifically acting on a small and immediate scale: light gaps or shade, primary or secondary forest, and scent preferences (which scents are more or less effective at attracting males from known scents). If bees chose to forage in light gaps opposed to closed canopy, or vice versa, this could reflect on how orchids were distributed. From previous reports, we expected to see more bees in light gaps (Schlising 1970). Furthermore, due to
orchids being more prevalent in primary forests, we expected to see more euglossine bees within primary forests, and more species of euglossines.

METHODS

We conducted studies on February 6-8, 2013, at Sirena Biology Station, Corcovado, Costa Rica. Without background knowledge of orchid bee scent preference, we used seven scents recommended by the American Museum of Natural History with the hope of attracting as many euglossine bees in total as possible to test habitat preference: benzyl acetate, cinnamon, eucalyptus, eugenol, peppermint, skatole, and vanilla. Each scent was prepared in a separate sample. Approximately 10 mL of ethanol was mixed with a few drops of a given scent; we used plastic Ziplock bags as elution devices (to yield an approximately constant elution rate). For a control, we used one plastic bag containing 10 mL of ethanol.

To determine if euglossine bees foraged more in light gaps versus shaded regions of the forest, we measured bee attraction to scents placed at six sites in the primary forest (three in light gaps; three in shade). Two sites of each type contained all eight scents while one site of each type contained partial arrays due to equipment limitations (site five, shaded: skatole, vanilla, eucalyptus, peppermint, eugenol, and benzyl acetate; site six, light gap: eucalyptus, skatole, peppermint and eugenol). The sites ranged from 60 m to 220 m apart, with distances calculated using GPS coordinates from a Garmin GPSmap 76CSx. Each site was observed for 20 minute intervals. For each bee that was attracted to the scents, we recorded the attracting scent and the coloration of the bee to quantify them as morphospecies. We also captured as many bees as possible with a net and measured intertegular distance (Greenleaf et al. 2007), the length of the entire bee, and the length of its tongue to evaluate whether coloration was a good indicator of morphospecies. Before release, we marked each bee with a permanent marker on a unique part of its to identify it in the event of a recapture. We estimated resource availability by measuring orchid density. At each site, we chose the closest large tree (DBH greater than 0.3 m) in each of four quadrants based on cardinal directions, and used binoculars to count the total number of orchids on all four trees.

To test for differences in euglossine bee distributions between primary and secondary forests, we chose three sites in each type of forest, and placed all eight scents at each site. Due to idiosyncrasies of our sampling, primary forest sites were observed for 40 minutes more than secondary forest sites. The primary forest sites were 94 m and 64 m apart and the secondary forest sites were 166 m and 180 m apart. The closest primary and secondary sites were 900 m apart. We repeated the same method as above for observations and measurements.

Data Analysis

To evaluate whether body color was a reliable means of recognizing bee species, we plotted the ratio of body length to tongue length for each morphospecies and visually assessed clumps within color morphs. Intertegular distances were also plotted, and were clumped together within morphospecies. We used Hurlbert’s PIE to test the species evenness of the euglossine assemblages collected in each treatment (gap vs. closed, primary vs. secondary). We observed differences between day 2 and 3, therefore also compared these two days to determine variation between days. We also compared abundance and species richness between sites. Although average number of bees per hour would have been a good measurement of bee attraction, we chose to present attraction as the total number of bees observed because bee activity changed so dramatically over time as to make average number of bees per hour an inaccurate metric of activity.

RESULTS

All morphospecies were tightly correlated in body to tongue ratio except for the green morphospecies, which had two clusterings, suggesting that there are at least two different species within the green euglossines (Table 1; Figure 1). We also measured intertegular distance and found values clustered within morphospecies. We recaptured one bee (at site 4, previously caught at site 3).
Within primary forest, we observed that a higher abundance of euglossines in light gaps (18) than shade gaps in closed forest (6; Appendix Table 1). Bee activity in light gaps tended to cease when the sun became obscured by clouds. We found six morphospecies in gap patches and six in shade patches. *Eulaema* orchid bees (black and yellow coloration) and the blue morphospecies were only observed in gap patches while the red bees were exclusively observed in shade patches. Evenness was similar between the two site types (closed canopy: 0.97; light gaps: 0.90).

On average we saw 19 bees per hour at secondary forest sites (52 total), while only 13 bees per hour at primary forest sites (64 total; Appendix Table 1). We observed seven morphospecies in each habitat type. Blue-yellow bees were only caught in the primary forest, while the green-yellow bees were more abundance in secondary (27) versus primary forest (1).

Bees were more attracted to the scent baits on the second day of sampling in both primary/secondary sites (7-12), with total bees observed increasing from 16 to 37 in primary forest sites and 22 to 42 in secondary forest sites (Table 2). Morphospecies richness also increased between sampling days. In primary forest sites, morphospecies richness increased from six to seven; green-yellow bees were only observed on day 2, whereas green-blue and green-red bees were only observed in day 3. In secondary forest sites morphospecies richness also increased from four to seven species as black-yellow, blue, and red bees were observed only on day 3. Evenness was similar between primary and secondary sites for both sampling days.

<table>
<thead>
<tr>
<th>Morphospecies</th>
<th>BL ±</th>
<th>TL ±</th>
<th>IT ±</th>
<th>BL:TL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black/yellow</td>
<td>27.2 ±</td>
<td>13 ±</td>
<td>7.7</td>
<td>2.09</td>
</tr>
<tr>
<td>Blue</td>
<td>14.1 ±</td>
<td>6.2 ±</td>
<td>4.0</td>
<td>2.29</td>
</tr>
<tr>
<td>Blue/yellow</td>
<td>23 ±</td>
<td>9 ±</td>
<td>4.0</td>
<td>2.56</td>
</tr>
<tr>
<td>Green*</td>
<td>13.5 ±</td>
<td>8.5 ±</td>
<td>3.3</td>
<td>1.59</td>
</tr>
<tr>
<td>Green/blue</td>
<td>14.9 ±</td>
<td>12.5 ±</td>
<td>3.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Green/red</td>
<td>11.8 ±</td>
<td>7.9 ±</td>
<td>3.5</td>
<td>1.49</td>
</tr>
<tr>
<td>Green/yellow</td>
<td>14.3 ±</td>
<td>7.6 ±</td>
<td>3.5</td>
<td>1.88</td>
</tr>
<tr>
<td>Red</td>
<td>12.8 ±</td>
<td>8.6 ±</td>
<td>3.5</td>
<td>1.48</td>
</tr>
</tbody>
</table>

*Potentially two morphospecies

Figure 1: Body to tongue length ratio plotted per morphospecies. All morphospecies are relatively tightly grouped except the green morphospecies, which seems to form two clusters, thus suggesting two morphospecies within this coloration. BL-Y = black-yellow, B = blue, B-Y = blue-yellow, G = green, G-B = green-blue, G-R = green-red, G-Y = green-yellow, R = red.
different morphospecies had different scent preferences (Table 2).

Orchid density varied widely between sites, ranging from 0 to 81 orchids per patch. The secondary forest had more orchids than the primary forest but had a large standard deviation (28 ± 46 and 5 ± 5 respectively), due to one tree at site 11 that had 81 orchids. Among all 140 observed bees, we saw four pollinaria, each from different orchid species.

**DISCUSSION**

Our results indicated a strong preference of euglossine bee for light gaps over closed canopy sites when foraging for scents. Increasing canopy cover is associated with decreased temperature and light in the understory (Balisky and Burton 1995), both of which can limit the foraging activity of male orchid bees (Armbruster and McCormick 1990), and may explain the trend we observed. Our data suggested that different euglossine communities forage in primary than secondary forest patches. Contrary to our expectations, primary forest gap patches contained fewer euglossines and less variety in morphospecies (Appendix Table 1). The greater total bee abundance observed was primarily driven by green-yellow bees, which appeared in higher numbers the second day of sampling. This result was likely conservative given that we sampled the secondary forest for 40 minutes less than the primary patches. In both forest patch types, total bee abundance and species richness increased substantially from day 2 to day 3. This supports Armbruster’s (1993) findings that bee visitation increases as scent traps are out longer since more bees can be attracted over time. Increased activity over the course of the study and the one recapture suggest that similar sampling over a longer period of time would yield a better sense of euglossine community structure.

Though it seems as though some scents, such as cinnamon and peppermint, were preferred less by all morphospecies and that morphospecies did have preferred scents (Table 2), our sample size was not large enough to draw conclusions. Different scent preferences among morphospecies would support the theory that specific euglossine species form relationships with specific plants (Gibert and Raven 1975). This specialization might enable multiple

<table>
<thead>
<tr>
<th>Morphospecies</th>
<th>Benzyl acetate</th>
<th>Cinnamon</th>
<th>Control</th>
<th>Eucalyptus</th>
<th>Eugenol</th>
<th>General</th>
<th>Peppermint</th>
<th>Skatole</th>
<th>Vanilla</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black/yellow</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Blue</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Blue/yellow</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Green*</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>15</td>
<td>7</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>45</td>
</tr>
<tr>
<td>Green/blue</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Green/red</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Green/yellow</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>14</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td>Red</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Sum</td>
<td>21</td>
<td>3</td>
<td>1</td>
<td>46</td>
<td>19</td>
<td>11</td>
<td>1</td>
<td>13</td>
<td>26</td>
<td>141</td>
</tr>
</tbody>
</table>
species with overlapping territories to share a single foraging area (Lundberg 1979). Given the incredible levels of species diversity and resource partitioning in the tropics, we suggest scent preference among morphospecies as an area of further study that could shed light on whether or not species diversity is correlated with niche specialization.

We found little relationship between orchid density and euglossine bee presence. This may be due to the large size of euglossine foraging territory (Roubik and Hanson 2004) and the small scale of our orchid density sampling. Because euglossines are able to cover large distances while searching for specific plants, orchid density in the immediate surroundings may not strongly affect the number of bees arriving at a scent trap. We suggest that future studies conduct a larger and more thorough orchid census in conjunction with use of scents to better test the relationship between male euglossine and orchid distribution.

Our study demonstrated that male euglossine most frequently forage in light gaps in both primary and secondary forests. We also identified different euglossine foraging assemblages of roughly the same evenness between the two forest types. Our results are indicate that both contribute to local biodiversity.

ACKNOWLEDGEMENTS
We would like to thank the staff of Sirena Biological Station for providing sustenance, Z. Gezon for his expertise, time, and help, and R. Chaves-Ulloa, Z. Gezon, and M. Ayres for their assistance in manuscript review.

AUTHOR CONTRIBUTIONS
All authors contributed equally

LITERATURE CITED
<table>
<thead>
<tr>
<th>Habitat</th>
<th>Day</th>
<th>Site Number</th>
<th>Number of 20 min observational period</th>
<th>Black/yellow</th>
<th>Blue</th>
<th>Blue/yellow</th>
<th>Green*</th>
<th>Green/blue</th>
<th>Green/red</th>
<th>Green/yellow</th>
<th>Red</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary forest, gap</td>
<td>2/6/13</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Primary forest, gap</td>
<td>2/6/13</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Primary forest, shade</td>
<td>2/6/13</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Primary forest, shade</td>
<td>2/6/13</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Primary forest, gap</td>
<td>2/6/13</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Primary forest, gap</td>
<td>2/6/13</td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Primary forest, gap</td>
<td>2/7/13</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Primary forest, gap</td>
<td>2/7/13</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Primary forest, gap</td>
<td>2/7/13</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Secondary forest, gap</td>
<td>2/7/13</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Secondary forest, gap</td>
<td>2/7/13</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Secondary forest, gap</td>
<td>2/7/13</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Primary forest, gap</td>
<td>2/8/13</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Primary forest, gap</td>
<td>2/8/13</td>
<td>8</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Primary forest, gap</td>
<td>2/8/13</td>
<td>9</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Secondary forest, gap</td>
<td>2/8/13</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Secondary forest, gap</td>
<td>2/8/13</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Secondary forest, gap</td>
<td>2/8/13</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>21</td>
</tr>
</tbody>
</table>

* Green morphospecies was shown to be two species
HONEST SIGNALLING FOR TERRITORY AND MATE INTERACTIONS IN STRAWBERRY POISON DART FROGS (*OOPHAGA PUMILIO*)

COLLEEN P. COWDERY, ELIZA W. HUNTINGTON, AND ELLEN T. IRWIN

Faculty Advisor: Matt Ayers

Abstract: Signal systems in nature serve a variety of purposes, including territory claims and advertisement for mates. Territorial signals generally seek to broadcast the presence of a male as a strong competitor; mating signals frequently advertise good genes or traits attractive to potential mates. The strawberry poison dart frog (*Oophaga pumilio*) employs both auditory and visual cues for these purposes; male calling and dorsal brightness are used to assess the male as a potential mate and competitor. As male *O. pumilio* are advertising themselves both as strong to competing males and as good mates to females, these signals should be honest indicators of body condition and aggression towards intruding males. To test our hypothesis we measured aggression, dorsal brightness, body condition and pulse rate of the call in *O. pumilio*. We found that neither visual nor auditory signals were related to body condition or aggressive behavior. However, there was a positive correlation between dorsal brightness and pulse rate of the frog calls. Because both brightness and low pulse rate are attractive to females, our results suggest that some constraint, physiological or otherwise, keeps males from exhibiting both characteristics at once.

Key words: female preference, mate-selection signaling, *Oophaga pumilio*, territorial signaling

INTRODUCTION

Organisms use signals for a variety of purposes, from communicating with conspecifics to warning away potential predators (Zuberbuhler et al. 1999). Signals relating to territoriality and mate selection are especially prevalent in nature. When guarding a territory, animals may use signaling to warn conspecifics not to approach; territorial disputes are energetically expensive, and displays may reduce the likelihood of conflict (Wagner 1992). In mate selection, members of the opposite sex may use cues to assess the fitness of a potential mate (Candolin 2003). Although territoriality and mate selection often have different endgoals, one signal may sometimes apply to both (Catchpole 1983).

Not all signals reliably indicate high fitness, however. In order for a signal to be considered “honest”, there must be a tight correlation between the trait and the individual’s fitness: for example, dewlap size in lizards can predict fighting ability (Vanhooydonck et al. 2005). In mate selection, cues preferred by one sex (such as bright coloration) may be honest if indicative of fecundity benefits or higher survival of offspring (Candolin 1999).

Male strawberry poison dart frogs (*Oophaga pumilio*) use both visual and auditory signals to attract females and maintain territory (Bunnell 1973; Zuluaga 2012). Males vocalize to reduce physical conflict, and both calls and dorsal brightness are related to male quality in territorial disputes (Crothers et al. 2010). Males may be less likely to engage in territorial disputes if warned by the brightness of a more fit male (Crothers et al. 2010). Calling and coloration signals are also used to attract females (Bunnell 1973; Maan and Cummings 2009), who prefer lower pulse rate and brighter color (Prohl 2003; Crothers et al. 2010). Because dorsal brightness and pulse rate relate to a male’s ability to defend a territory, these signals may indicate both male quality and territory quality. Therefore, female *O. pumilio* may use territory quality to choose mates because territory can influence reproductive success (Donnelly 1989).

Females should be selecting for desirable traits in mates to increase the fitness of their offspring (Candolin 1999). If brightness and pulse rate are honest signals of good genes, then determinants of mate quality such as body condition and territorial aggression in males should be positively correlated with female preferences (e.g. brighter frogs). If brightness and pulse rate are not related to male condition, then sexual selection may be working against natural selection, as females are selecting for traits that do not contribute to male survivability. In this case, males could be exploiting a sensory
bias in order to both minimize territorial conflict and gain greater access to mates by taking advantage of female *O. pumilio*’s innate preference for specific colors or sounds, even if they are not honest signals of good genes.

**METHODS**

We located male *O. pumilio* along trails and clearings in La Selva Biological Reserve, Heredia, Costa Rica on February 15-18, 2013. We located individual males by their calls, and whenever possible recorded their call using Olympus dictaphones. After recording an individual male, we placed the dictaphone between 0.5 and 1 meter from the frog and played a male *O. pumilio* call that we recorded February 14. To gauge territoriality for the previous two tests, we took notes on aggressive behavior for the three minute trial, and scored each frog on a scale of increasing aggression: 0 = no response, 1 = call response, 2 = movement towards the dictaphone, 3 = both calling and movement. For cases in which we were unable to obtain a calling before the trial, we recorded the individual’s response call and noted it as ‘post-aggression.’ After the aggression trial, we captured the frog and measured snout-vent length and weight, and used a Canon Digital Rebel XT to photograph its dorsal area.

To determine differences in brightness between frogs, we calculated dorsal brightness using GIMP image editing software. Using the RGB histogram, we determined the mean red value of the dorsal area and divided it by the value of pure red to obtain a percentage deemed “brightness.” To understand the auditory signal of the frogs, we analyzed the pulse rate (number of discrete pulses per second at the peak of the call) of the calls with RavenLite and Praat audio analysis software. For several frogs, we had obtained both pre- and post-aggression trial calls. We examined the pulse rate of all paired recordings to determine if we could compare pre- and post-aggression calls within our main dataset.

**Statistical Analysis**

We ran a regression of weight as a factor of length and saved the residuals to be used as ‘body condition.’ To understand the relationships between body condition, perceived territoriality, brightness and pulse rate of the calls, we ran a series of correlation analyses. We tested brightness in relation to body condition and aggressive behavior; pulse rate versus body condition and aggressive behavior; and finally the correlation between brightness and pulse rate. We used JMP 10.0 for all statistical analyses; all assumptions were met prior to testing.

**RESULTS**

We found a significant relationship between dorsal brightness and pulse rate (r=0.45, $F_{1,19}=4.79$, $P = 0.041$; Figure 1). We found no relationship between body condition and signal aspects; there was no correlation of body condition with brightness ($r=0.18$, $F_{1,26}=0.83$, $P = 0.37$) or with pulse rate ($r=0.23$, $F_{1,19}=0.98$, $P = 0.33$). Furthermore, there was no relationship between aggressive behavior and brightness ($r=0.26$, $F_{1,26}=1.82$, $P = 0.19$) or pulse rate ($r=0.20$, $F_{1,19}=0.76$, $P = 0.39$). For the few individuals for which we recorded both pre- and post-aggressive trial calls, we determined qualitatively (we did not have enough data to perform statistical analyses) that the two calls differed in both pulse rate and frequency enough that we could not compare the two types.

![Figure 1. Pulse rate was positively correlated with dorsal brightness of *Oophaga pumilio*. Data were collected 15-18 February 2013 at La Selva Biological Station, Heredia, Costa Rica.](image-url)
DISCUSSION

Our results suggest that body condition is not correlated with dorsal brightness, pulse rate, or aggressive behavior. Previous studies also found no correlation between body length, mass and any call aspects (Prohl 2003). Additionally, past studies have found that in *O. pumilio* body length and mass were not correlated with success in aggressive encounters (Graves et al. 2005). Thus, body condition may not be a physical advantage within the species, and therefore is not necessarily a measure of male quality. In other species of frogs, size is not associated with mating success because females choose mates closer to their own size (Graves et al. 2005). If this is also the case in *O. pumilio*, it follows that size might not be correlated with brightness, which is a sexually-selected trait (Maan and Cummings 2008). Because body condition may not be a good measure of male quality, pulse rate and brightness should not necessarily be associated with body condition. It could also be that pulse rate and brightness indicate some other measure of male quality that females are selecting for, such as toxicity.

We found no relationship between aggression and either brightness or pulse rate. Therefore, pulse rate and dorsal brightness may not be honest indicators of male quality in territorial disputes. Male *O. pumilio* may use vocalizations and bright color as a more effective way of recognizing an occupied territory. If so, this could minimize physical conflict by making intruders aware of potential conflict with current territory holders.

Though we found no correlations between body condition and either pulse rate or brightness, we did find that dorsal brightness was positively correlated with pulse rate. Female *O. pumilio* have preferences for high brightness and low pulse rate in males (Prohl 2003). Exhibiting “pure” red brightness is a signal of both higher toxicity, an aposematic trait that is advantageous to the survival of the male, and of heightened carotenoid levels indicative of good foraging ability, which is attractive to females (Brusa 2012; Maan and Cummings 2012). Prohl (2003) found that pulse rate was negatively correlated with age, which may be an indicator of viability. Interestingly, we found that pulse rate may differ in *O. pumilio* after individuals are agitated by the sound of an intruding male; our data were inconclusive but we believe that pulse rate variability deserves further study as current literature states that pulse rate is not as variable in a single individual male as it is between males (Prohl 2003).

Though brightness and low pulse rate are both attractive to females, we found no males with both of these traits, suggesting that some constraint, physiological or otherwise, keeps males from exhibiting both characteristics. A possible explanation is that older frogs with low pulse rates are unable to forage as well as young frogs. Because dorsal brightness in *O. pumilio* is dictated by diet, it may be indicative of both carotenoid levels and toxicity, both of which are gained through foraging (Santos et al. 2003). Thus, frogs with lower pulse rates would be unable to also have bright dorsal coloration. Alternatively, older frogs may not need to expend energy to obtain carotenoids for brighter coloration. They may already have good territory, enabling them to compete with younger, brighter males.

We found that though body condition does not have a noticeable relationship with either brightness or pulse rate in *O. pumilio*, these visual and auditory signals have a positive correlation with each other. As low pulse rate and high brightness are both preferred by female frogs, this correlation shows that males seem to be constrained in some way from achieving both preferred traits simultaneously, suggesting the presence of some trade-off or environmental restriction at play. Trade-offs in signaling are frequently imposed by either organism physiology or by signal exploitation by competitors or predators (Kotiaho 2001). On top of this, female preference in this system is being pulled by two non-coincident traits—one a function of age, and one a function of diet. Signals do not always point in the same direction; when they do not, a given signal may overpower or lessen an opposing signal (Kotiaho 2001). In systems where multiple signal types, meanings, and recipients are in play, the resulting web can be complex and difficult to disentangle. Greater knowledge of these signal webs can improve our overall understanding of signaling within and among species.
ACKNOWLEDGEMENTS
We thank the staff of La Selva Biological Station for providing housing and sustenance, and thank Z. Gezon, R. Chaves-Ulloa, and M. Ayres for their time and assistance in manuscript review.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED


ISLAND BIOGEOGRAPHY: ARE HELICONIAS ISLANDS?

TYLER BILLIPP AND SETH A. BROWN

Faculty Editor: Matt P. Ayres

Abstract: The factors influencing species richness are of broad relevance to ecology. The equilibrium island biogeography theory posits that island species richness is strongly influenced by island size as well as distance from a source population. Subsequent studies have found that caves, patches of isolated forest, and even host plants can be understood in terms of the island biogeography model. In this study, we analyzed the degree to which H. wagneriana patches represent ecological islands by studying the invertebrate communities housed within their fluid-filled bracts. We also analyzed differences in community composition between bract age classes. We found a weak positive trend between species richness and patch area, consistent with the island biogeography model, but no relationship between isolation distance and species richness. Furthermore, there was no significant difference in the community composition among the three bract age classes. The scale at which Heliconia patches occur seems too small relative to dispersal abilities of the populations that inhabit their bracts to have the characteristics of island communities.

Key Words: Island biogeography, Heliconia, bract

INTRODUCTION

Species richness influences the stability and productivity of biological communities (Tilman et al. 1997, Knops et al. 1999). Thus, factors affecting species richness are of fundamental interest in ecology. MacArthur and Wilson (1967) demonstrated that island species richness is strongly correlated with island’s area and distance to the mainland. Their theory on island biogeography states that the species richness on an island is defined as the intersection of extinction and immigration rates, which vary with the island’s area and distance from the mainland. For example, communities on small islands far from the mainland are generally smaller and less diverse than communities on large islands close to the mainland due to higher extinction rates and lower immigration rates.

The basic tenets of island biogeography theory can also apply to non-island systems where organisms live in spatially distinct habitats, such as patches of forest isolated by anthropogenic change (Bolger et al. 1991). The model has even been applied to individual host plants (Janzen 1968). For all of these symbolic islands, the species-area relationship is apparent, but the distance effect appears to be less influential toward species richness (Culver et al. 1973, Seifert 1975).

The fluid-filled floral bracts of some species of Heliconia support diverse communities of aquatic invertebrates and can constitute some of the few patches of standing water in Neotropical forests (Seifert 1974, 1982). Bract invertebrates, mainly fly, mosquito, and beetle larvae, rely on Heliconia for food and shelter during development, leaving after they pupate as terrestrial or flying adults. As a Heliconia inflorescence grows, more bracts open with time, allowing for temporal niche partitioning by bract (Richardson and Hull 2008). Older, larger bracts are dominated by mosquito larvae, while younger, smaller bracts contain fly and beetle larvae (Seifert 1982). Since Heliconia most often reproduce from rhizomes, inflorescences are patchily distributed throughout the forest (Seifert 1982).

In this study we tested the applicability of the island biogeography model to patches of Heliconia wagneriana by examining the aquatic insect communities within bracts. We also examined variation in community composition by bract age. We predicted that species richness would increase with Heliconia patch size but be unaffected by distance (Seifert 1975) due to the dispersal abilities of adult insects. Additionally, we predicted that community composition would differ systematically among bracts of different ages.

METHODS

We sampled nine H. wagneriana patches at La Selva Biological Station, Heredia province, Costa Rica on 16-17 of February, 2013. We
systematically chose patches within 210 m of each other along the Sendero Arriera-Zampopa. We selected three inflorescences per patch for sampling: the middle and the two outermost inflorescences per patch. When there were fewer than three inflorescences present, we sampled them all in the patch. We extracted the fluid held within the top, middle, and lowest bracts from each focal inflorescence using a 1.5 oz turkey baster and examined all 60 samples under a dissecting microscope. If an inflorescence contained an even number of bracts, the lower of the two middle bracts was sampled. All insects were identified to order or family and then sorted by morphospecies.

We also recorded total number of inflorescences and bracts within a patch and recorded distance (m) between the two farthest inflorescences for an approximation of patch density, (patches were typically arranged in a line). We used total bracts per patch as the equivalent to island area to analyze species-area effects. We recorded the latitude and longitude of each patch with a Garmin GPSmap 76CSx and used boulter.com to analyze the interpatch distances. We then calculated the mean isolation distance for a patch as the average distance from that patch to all other patches. The last three patches were excluded from the distance data because at that site we only sampled a subset of the total patches and therefore the mean isolation distance would not have been representative of the distance effect.

Statistical Analysis
All data were analyzed using JMP 10.0 software and all assumptions for tests were met. To examine if *H. wagneriana* patches exhibited similar species-area relationships to true islands, we used a simple linear regression with ln(species richness) as a function of ln(total bracts). We performed a principal components analysis (PCA) of morphospecies at level of bract. We analyzed the resulting values for each bract of the 1st and 2nd axes of the PCA with a general linear model that included patch, plant nested within patch (as a random effect), and bract age class (1 – 3 for young to old) as a continuous variable. We applied the same model to analyze the (square root transformed) abundance of the three most abundant morphospecies: Culicidae morph 1, Syrphidae morph 1, and Chironomidae morph 1.

RESULTS
We found a positive trend between ln(total bracts) and ln(species richness), which was consistent with the island biogeography model although the relationship was not significant \( r^2 = 0.23, P = 0.19 \). However, we found no negative correlation between mean isolation distance and species richness which is inconsistent with the island model \( F_{1,4} = 0.05, P = 0.83 \). The GLM’s were all insignificant, indicating that neither the morphospecies abundance nor composition of morphospecies varied among patches or among bracts (all \( F < 3.70, \) all \( P > 0.05 \)).

![Figure 1. The non-significant positive trend between total bracts and invertebrate species richness within patches of *H. wagneriana* \( (r = 0.23, P = 0.19) \).](image)

DISCUSSION
Although our results suggest a trend between patch area and species richness, we conclude that the conventional model of island biogeography does not hold for patches of *H. wagneriana*, for several reasons. First, we did not find a negative relationship between mean patch isolation distance and species richness. The lack of correlation indicates that species richness in *H. wagneriana* patches does not vary with distance between patches. It is possible that *Heliconia* patches are not sufficiently isolated for immigration probabilities to be reduced to
distance-mediated random chance, the way the island biogeography model necessitates. The dispersal ability of flying adult insects may largely eliminate dispersal limitations at this scale.

Second, we found no significant variation in community compositions between patches. If *H. wagneriana* patches were truly islands, one would expect differences in community composition among patches, and that closer patches would be more similar in their insect communities due to the increased probability of dispersal between the patches. Since we did not see more community overlap in adjacent patches compared to distant patches, larger patches likely achieved greater species richness because their area supported higher colonization success rates. Thus, larger *H. wagneriana* patches were not found more often by dispersing insects, but they were likely colonized more due to greater overall habitable space and greater niche redundancy.

Likewise, we found no significant variation in community composition between bract age classes. Our findings conflict with those of Richardson and Hull (2008) who found clear patterns in *H. caribaea* bract community composition with respect to age. The discrepancy might be due to fundamental difference in the biology of the two *Heliconia* species and the insect communities they are capable of supporting, but more cannot be said without more comprehensive censusing of *H. wagneriana* bracts.

The island biogeographic model is less applicable to *Heliconia* patches than some systems because of their differences in scale. Since species extinction does not occur at the individual *Heliconia* patch level, but at the level of the larger geographic community, no equilibrium can be reached between immigration and extinction rates. Allele frequencies within patch species communities might be a more suitable level for equilibrium analysis. Further studies could test if populations are confined to patches of *H. wagneriana*. Less genetic variation would suggest a tendency for adult insects to recolonize (i.e., lay eggs) in their natal patch rather than to migrate to other patches. Such a trend could be indicative of either dispersal limitations on an individual level or preference for some component of the natal environment.

The species richness of an island is largely determined by its size and distance to the mainland. Beyond terrestrial islands in seas of water, however, there exists a wide array of spatially distinct habitat patches in which principles of the island biogeography model might be applicable. Our results suggest that *H. wagneriana* does not act as an island in terms of species richness, but may yet act as a genetic island. In addition, our results highlight the importance of further research into the circumstances under which the island biogeography model does and does not apply.

Better understanding of the island biogeography model and its applications can inform conservation decisions and population management.

**ACKNOWLEDGEMENTS**

We thank Professor Matt Ayres, Zak Gezon, and Ramsa Chaves-Ulloa for their help with the statistical analysis and interpretations. All authors contributed equally.

**LITERATURE CITED**


MITE Proctolaelaps kirmsei Negatively Affect Hamelia Patens and Its Hummingbird Pollinators?

Amelia F. Antrim, Jimena Diaz, Samantha C. Dowdell, Maria Fransisco, Emilia H. Hull

Faculty Editor: Matt Ayres

Abstract: Costs and benefits are important to interspecific interactions because all players seek to maximize their gains. In plant-pollinator mutualisms, plants rely on organisms such as bees, wasps, and hummingbirds for pollination, while pollinators depend upon rewards such as nectar. However, attracting pollinators may come at the cost of acquiring non-pollinating parasites. The parasitic mite Proctolaelaps kirmsei relies on hummingbird pollinators to disperse among Hamelia patens trees. We examined the effect of hummingbird visitation on average mite abundance in H. patens trees, as well as the effect of mite abundance on H. patens reproductive success and number of hummingbird repeated visits. Mite abundance increased with the number of hummingbird visitors, implying that hummingbird visitation was a driver of P. kirmsei presence on H. patens. However, mite presence did not affect fruit production or the number of repeated visits per hummingbird, suggesting that mites may not deplete nectar resources sufficiently to affect H. patens female reproductive success or hummingbird foraging. The relationship between H. patens, P. kirmsei, and hummingbirds provides an example of an apparently stable parasitic relationship. Evaluating the dynamics of sustained parasitism may shed light on how these interactions persist in natural systems.

Key words: Hamelia patens, Proctolaelaps kirmsei, phoretic mites, plant-pollinator interactions

Introduction

Costs and benefits are important to interspecific interactions because all players seek to maximize their gains. In mutualisms, the benefits of maintaining a relationship between two parties outweigh the costs (Bronstein 2001). Although one or both parties may experience negative aspects of the relationship, the net reciprocal benefits allow the mutualism to persist over time.

In plant-pollinator mutualisms, plant species rely on mobile organisms such as bees, wasps, and hummingbirds for pollination, while these pollinators depend upon rewards provided by the plant. Plants have evolved many mechanisms to attract and reward these pollinators, such as volatile compounds that emit attractive scents or nectar high in sugar content (Pellmyr and Thien 1986). However, plants’ adaptations to entice pollinators may also attract non-pollinating parasitic organisms such as mites, ants and beetles (Colwell 1973). Plants that rely on pollinators might therefore encounter a trade-off between attracting pollinators and avoiding parasites. The degree to which a parasite affects host fitness often depends on the life history and identity of the key players involved (Maloof and Inouye 2000).

One such parasitism involves the mite Proctolaelaps kirmsei, which takes advantage of the relationship between the tree Hamelia patens and its hummingbird pollinators. P. kirmsei is dependent on not only H. patens as a food source but also the hummingbirds themselves as its primary means of dispersal (Colwell 1973). Mites consume large quantities of pollen and up to 40% of available nectar, depleting the plant’s nectar resources (Colwell 1995). By feeding on the plant’s pollen and nectar, mites decrease the quantity of male gametes available for dispersal as well as the nectar rewards for legitimate pollinators. This adds to the burden on H. patens by forcing the trees to produce extra amounts of nectar to compensate for losses (Colwell 1995). The cost of parasitism in H. patens is therefore inevitably linked to the benefits of attracting hummingbird pollinators.

As H. patens is pollen-limited (Burkle et al. 2007), pollinator interactions can influence female reproductive success and therefore fruiting (Harder and Aizen 2010). We investigated whether mite presence is related to hummingbird visitation and whether the presence of P. kirmsei affects the female reproductive success of H. patens. As P. kirmsei are entirely dependent on hummingbirds for dispersal, trees that regularly attract more...
hummingbirds are more likely to later support larger mite populations. However, if the presence of mites depletes pollinator rewards, hummingbirds might be less likely to forage on a tree with mites, which would decrease cross-pollination and potentially female reproductive success. We would then expect decreased pollinator visitation and fruiting in plants with more mites. We also investigated whether mite presence affects repeated visitation on one tree. Low nectar levels could increase repeated visitation and therefore pollination as hummingbirds would be forced to visit more flowers on a plant to gain the same amount of nectar (Colwell 1995).

METHODS
We conducted our study on February 16-18, 2013 at La Selva Biological Station, Costa Rica. To determine the effects of *P. kirmsei* on the reproductive success of *H. patens*, we haphazardly chose four *H. patens* trees to observe on February 16 and 17, and six trees on February 18. We observed each tree for 30 minutes between 6:00-7:30 am. We recorded the number of hummingbird visits per tree, regardless of whether a hummingbird returned multiple times. We also recorded the number of repeated visits per bird, defined as the number of inflorescences a hummingbird visited before flying away. We did not count visits to multiple flowers on the same inflorescence. Later in the morning (9:00-11:30 am), we haphazardly chose 5 branches from each tree and recorded the number of inflorescences and infructescences on each branch as a measure of reproductive success. We then removed one inflorescence from each of the selected branches and counted the total number of flowers and open flowers on the inflorescence to investigate mite distribution and abundance within inflorescences. Afterwards, we haphazardly picked three open flowers (or as many as were available if fewer than three were open) and counted the number of mites were in each selected flower. We were unable to collect nectar or conduct pollen counts due to logistical constraints.

Statistical Analysis
To determine how mite abundance varied as a function of hummingbird visitation, we used simple linear regression. We log10 transformed number of hummingbird visits and number of mites per flower to achieve normality. To determine how *H. patens* female reproductive success varies as a function of mite abundance, we used simple linear regression, calculating the mean ratio of infructescences to inflorescences on a tree as a proxy for female reproductive success. Finally, we investigated how mite presence influenced hummingbird foraging on one tree, using regression analysis to determine how mean repeated hummingbird visits varied as a function of mite abundance. We log10 transformed mean repeated visits to achieve normality. All analyses were conducted using JMP 10.0 statistical software and assumptions for linearity and normality for all tests were met.

RESULTS
Mean number of mites per flower increased with hummingbird visits per tree ($r^2 = 0.36, P = 0.02$, Figure 1). We found no relationship between mean mites per flower and *H. patens* reproductive success ($r^2 = 0.04, P = 0.48$, Figure 2). We also found no relationship between mean hummingbird visits to a tree and mean mites per flower on an inflorescence ($r^2 = 0.05, P = 0.46$, Figure 3).

![Graph showing the relationship between log10 (hummingbird visits per tree) and log10 (mites per flower).](image-url)
DISCUSSION
Mite abundance increased with the number of hummingbird visitors, implying that hummingbird visitation was a major determining factor for *P. kirmsei* presence on *H. patens*. Therefore, as hummingbird visitation increases, the probability of a tree being visited by a bird carrying mites also likely increases. The trade-off between pollination and mite presence could affect the fitness of both pollinator and plant.

The presence of mites did not appear to negatively impact *H. patens* female reproductive fitness or hummingbird foraging strategy. Mite presence had no effect on host plant fruit production, suggesting that resource loss as a result of mite foraging may not be large enough to reduce fruiting, although seeds per fruit may have been affected. Mean number of inflorescences per tree visited by each foraging hummingbird was also unaffected by mite abundance, implying that the parasitism does not influence hummingbird foraging. The natural history of *H. patens* might contribute to its ability to reproduce in the presence of mites; the population of mites on an individual inflorescence could be limited by the short life of *H. patens* flowers (Lara and Ornelas 2002). Short-lived flowers may therefore be an adaptation to limit the proliferation of parasites within a single flower. Further study on how mites affect reproduction and hummingbird pollination could track inflorescences over time rather than using infructescence to inflorescence ratios as a snapshot of the plant’s reproductive success. Additionally, future studies could incorporate nectar measurements as well as seed and pollen counts in order to determine the nature of the relationship between mite abundance, flower resource depletion, and reproductive success.

The relationship between *H. patens*, *P. kirmsei*, and hummingbirds provides a potential example of a parasitic relationship that has been sustained without forcing any party to alter its life history strategy. Parasitic and predatory interactions can lead to extinction. Evaluating the dynamics of sustained parasitism may shed light on how these interactions persist in natural systems.
ACKNOWLEDGEMENTS
We thank the staff of La Selva Biological Research station for sustenance and R. Chaves-Ulloa, Z. Gezon, and M. Ayres for their assistance and feedback.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
Sharing is Caring: Foraging Benefits in Mixed-Species Flocks of Toucans and Oropendolas

Gillian A. O. Britton, Kali M. Pruss, Molly R. Pugh, Elisabeth R. Seyfert, and Victoria D. Stein

Abstract: Acquisition of food resources is a driver of interspecific interactions within biological communities. Organisms can coexist by navigating interspecific interactions involving resource acquisition by either competing for or partitioning limited resources. The coexistence of bird species in mixed-species flocks may illustrate interspecific interactions at work and clarify the nature of these relationships. Toucans, araçaris, and oropendolas have been observed to form mixed-species flocks of varied size and species composition near La Selva Biological Station, Costa Rica. Three possible explanations for Ramphastidae-Icteridae mixed flocks are (1) increased predator detection and avoidance, (2) unintentional association due to shared resource requirements, and (3) intentional association for one or several species’ benefit. Foraging birds were in significantly larger flocks than non-foraging birds, implying that large flock size increases foraging success. Toucans and oropendolas were less likely to associate when foraging for plant-based resources but more likely to associate when foraging for small animal prey, suggesting benefits for one or both groups. Our study suggests that mixed flocking is driven, at least in part, by the foraging behavior of one species contributing to the success of another.

Key words: Mixed-species flocks, Psarocolius wagleri, Psarocolius montezuma, Pteroglossus torquatus, Ramphastos sulfuratus, Ramphastos swainsonii

Introduction

Food resource acquisition is a prominent driver of the interspecific interactions upon which organismal communities are built. To coexist, different species with similar diets in these communities must compete for or partition food resources such that each retains a portion sufficient for survival (Schoener 1974). Organisms may partially exclude one another physically from resources through defense and aggression, or they may create niches by dividing resources and resource acquisition temporally, spatially, or behaviorally (Schoener 1974).

The coexistence of bird species in mixed-species flocks may illustrate interspecific interactions at work and clarify the nature of these relationships. Flocking birds may choose to closely associate with one another in mixed flocks for many reasons, including lessened risk of predation and reduced energy expenditure when locating patchy resources (Graves 1993). However, not all species necessarily benefit by the presence of other species in the flock. Some relationships may be more commensal rather than mutual. In addition, to capitalize on potential benefits by associating closely with other species, flock members must successfully reconcile their individual resource needs and may therefore demonstrate niche partitioning (Latta and Wunderle 1996).

Toucans, araçaris, and oropendolas have been observed to form mixed-species flocks of varied size and species composition near La Selva Biological Station, Costa Rica. The Ramphastidae—Keel-billed Toucan (Ramphastos sulfuratus), Chestnut-mandibled Toucan (Ramphastos swainsonii), and Collared Araçari (Pteroglossus torquatus)—feed on fruit, insects, small vertebrates, eggs, and nestlings (Schulenberg 2013). The Icteridae—Montezuma Oropendola (Psarocolius montezuma) and Chestnut-headed Oropendola (Psarocolius wagleri)—feed on fruit, nectar, and insects (Schulenberg 2013). The diets of both groups include fruits and insects but differ in other food items, which may provide insight into how the different bird families forage together and apart.

Our study investigated the nature of...
relationships between the species in Ramphastidae-Icteridae mixed flocks by observing their association when engaged in different foraging behaviors. Three possible explanations for the occurrence of mixed flocks are (1) lower predation risk because more individuals are present to watch for predators (mutualism), (2) unintentional association due to shared resource requirements (competition), and (3) intentional association for one or more species’ foraging benefit (commensalism or mutualism). First, if the birds are flocking for safety from predators, we should find these flocks mixing at all times, regardless of their activity. Even if susceptibility to predators is higher while foraging because individuals are more distracted, toucans and oropendolas ought to be seen together regardless of their foraging food type. Second, if toucans and oropendolas are observed foraging for both fruits and insects in proximity to one another, it is possible that they happen to use the same resource at the same time and do not intentionally interact. Both resources may be sufficiently plentiful for the birds; our study took place during the Costa Rican dry season when both insects and fruits are plentiful (Janzen 1973, Chang 2008). In this case, since toucans do not eat nectar, we would not expect to find toucans at nectar resources where we might find oropendolas, but we would find them together while foraging for fruit or insects due to shared diet. Third, if toucans and oropendolas are found associating with one another only when foraging for insects and small vertebrates it would point to purposeful aggregation; foraging in mixed groups may stir up more prey and increase foraging success, resulting in mutual or commensal benefit to a higher number of individuals. Neither toucans nor oropendolas would benefit from foraging together for fruit; foraging in mixed groups may not help individuals to obtain more fruit but instead increase competition, so mixed-species flocks should not be observed at fruiting trees.

METHODS
On 15-18 February 2013, we searched the forest in and around the La Selva Biological Station and observed both single-species and mixed-species flocks of toucans, araçaris, and oropendolas. In total, we spent ~120 person-hours in the field, with a total of 61 encounters with either flocks or individuals. Encounters were separated either spatially or temporally, and we assumed their independence based on observations of flock boundaries and flight directions. Each time we encountered birds of either species, we counted the number of birds of each species present, documented their main activities (flying, perching, foraging, calling), and the type of tree(s) most birds were in (identified to plant family as well as whether it was fruiting or flowering). The specific locations and type of the different flocks we encountered can be found in Appendix 1.

Statistical Analyses
To determine whether foraging behavior was related to flocking behavior, we used a two-sided unequal variance t-test comparing total flock size between foraging and non-foraging flocks. To test how foraging flock composition changed based on foraging food type, we ran a chi-square analysis of the presence or absence of toucan species in flocks of oropendolas against the food type for which oropendolas were foraging (plant or animal food source). To test for the presence or absence of oropendolas in a toucan flock based on the food type for which toucans were foraging (plant, animal, or both), we ran a chi-square analysis. JMP 10.0 software was used for all analyses and data met all assumptions of chi-square and t-test.

RESULTS
Oropendola-only flocks comprised 43% of all sightings, mixed flocks comprised 31%, and toucan-only flocks comprised 26% of our encounters. We found huge variation in ratios of species and flock sizes. In particular, large non-mixed flocks of oropendolas were observed in Erythrina poeppigiana, a nectar-producing tree, with an average of 34 ± 16 oropendolas (mean ± 1 S.E.) and a maximum flock size of 111. The largest mixed flock we found contained 23 toucans and 12 oropendolas, but we observed large variation in flock size (one individual to 111). Most mixed-species flocks were dominated by oropendolas and contained five or fewer toucans regardless of the number of oropendolas. When toucans and oropendolas were together, Chestnut-mandibled Toucans (mean ± 1 S.E. = 2 ± 1) and Montezuma Oropendolas (9 ± 3) were observed most often. Toucans tended to be in the middle or rear of a mixed flock and often appeared to be
following oropendolas.

Total flock size of oropendolas and toucans was also significantly greater when birds were foraging (mean ± 1S.E. = 25 ± 4; Fig. 1) than when they were not (4 ± 3, $t_{21} = 3.37, P = 0.003$). Toucans were significantly more likely to be mixing in a flock with oropendolas when oropendolas were foraging for invertebrates than when oropendolas were foraging for nectar or fruit (chi-square = 5.67, $P = 0.017$; Fig. 2). Additionally, the mixing of the two bird families was significantly different when compared against the food for which toucans were foraging (fruit, invertebrates or vertebrates, or both) (chi-square = 5.55, $P = 0.063$; Fig. 3). Oropendolas were present in every flock of toucans foraging exclusively on invertebrates or small vertebrates, in 50% of toucan flocks foraging on both animals and fruit, and in no toucan flocks foraging exclusively on fruit.

**DISCUSSION**

Our results suggest that predator avoidance was not a primary cause of mixed flocking of toucan and oropendola species. If predator avoidance was driving mixed flock occurrence, we would have expected to find mixed species in large groups whether the birds were foraging or not and regardless of whether species were targeting food items shared by both bird families. However, bird flocks were larger when foraging than when traveling, calling, or perching (Fig. 1), implying that the large flocks convey some sort of foraging advantage to the toucans and oropendolas. Birds were also not observed together when foraging exclusively on non-shared resources: oropendolas feeding on nectar were never joined by toucans. Additionally, toucans and oropendolas are large and have few predators (Schulenberg 2013), so predation is unlikely to be a major cause of mixed-species flocking.

Our results further suggest that the occurrence of toucan and oropendola mixed flocks is related to the food type for which birds were foraging but was not an unintentional association due to shared resource requirements. The two families did not associate as frequently when foraging for nectar (commonly consumed only by oropendolas) or fruit (a shared resource) as they did when foraging for insects (Fig. 2 and 3). Toucans were never found in mixed-species flocks with oropendolas if oropendolas were foraging for nectar or fruit (Fig. 2). Conversely, every time we observed toucans foraging exclusively for insects and small vertebrates (i.e. not near or in fruiting trees) they were in mixed flocks with oropendolas, but the two bird families were not observed together when toucans were foraging exclusively for fruit (Fig. 3).

Our results then suggest foraging benefits as a cause of interspecies flocking. The addition of more birds, regardless of species, to form larger flocks may enable faster localization of food.
sources in patchy areas (Ward and Zahavi 1973). While the large foraging flocks we observed at nectar-producing trees may have been due to the relative ease of access to these stationary and highly aggregated resources, flocks were also larger when searching for mobile prey and may be due to benefits of foraging in larger numbers for less aggregated food sources (Fig. 1).

Toucans may derive additive benefit from mixed flocking due to the insect foraging activity of oropendolas. Oropendolas and toucans overlap in some areas of their diet, though not all; while oropendola animal prey consists mostly of small invertebrates, in addition to invertebrates, toucan species feed on small vertebrates such as lizards and bird nestlings (Stiles and Skutch 1989). A moderate amount of disturbance is advantageous for active foraging; it has been shown that some avian predators can use disturbance and prey escape responses to increase their hunting success (Jablonski 1996, Jablonski 1999) and that different foraging techniques (rapid movement or slow observation) can partition the prey that is flushed or discovered by avian predators (Robinson and Holmes 1982). While all birds create some disturbance with their movements, oropendolas were particularly active foragers and may have stirred up more prey items than toucans, increasing toucans’ foraging success without increasing their energetic output, as observed in temperate and tropical mixed-species flocks by Robinson and Holmes (1982) and Munn (1986). We observed toucans to be more slow and deliberate in foraging, which may show how toucans conserve energy by following oropendolas and consuming disturbed animal prey, potentially including both the invertebrates that escape oropendolas and the small vertebrates that the oropendolas disturb but do not consume.

It is unlikely that oropendolas are negatively affected by mixed-species flocking with toucans. Instead, our study provides evidence for a mutualistic relationship between toucans and oropendolas. Even though toucans are nest predators and thus pose a potential threat to oropendola nestlings (Stiles and Skutch 1989), we observed no defensive or aggressive behavior between the two bird families. A lack of aggression initiated by oropendolas may indicate that when oropendolas are foraging, there is little threat of nest predation by toucans. The absence of conflict may also suggest minimal direct competition with toucans. Rather, oropendolas may benefit from the presence of toucans, both because of the greater search capabilities of larger flocks and because toucans exclude smaller birds that may compete with oropendolas for nectar or invertebrates (Dolby and Grubb 1998).

Our study shows that mixed-species flocking is at least partially driven by the foraging behavior of one species contributing to the success of another in either a commensal or mutual relationship. The tight relationships between particular bird species in the Neotropics demonstrate a model system for exploring the nature of overlapping foraging niches. Shared niches may in some cases lead to competitive exclusion; however, when niches are partitioned in a way that reduces direct competition and foraging costs, interspecific aggregation may provide benefits to one or more species and favor long-term coexistence.

ACKNOWLEDGEMENTS
We thank the staff of La Selva Biological Station for accommodation and sustenance, the guides for
aid in locating birds and describing bird behavior, the students of the Dartmouth Study in Tropical Biology for aid in locating birds, and Matt Ayres, Zak Gezon, and Ramsa Chaves-Ulloa for their guidance.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
Appendix 1. Encounters with toucans and oropendolas in the La Selva Biological Reserve on 15-18 February 2013.
FRIENDS WITH BENEFITS:  
DOES SCHOOLING BEHAVIOR ENHANCE FORAGING IN BLUE TANG, *ACANTHURUS COERULEUS*, DURING INTERACTIONS WITH TERRITORIAL DAMSELFISH?

SAMANTHA C. DOWDELL, MARIA ISABEL REGINA D. FRANCISCO, EMILIA H. HULL, AND MOLLY R. PUGH

Faculty Editor: Brad Taylor

Abstract: The advantages of grouping behavior have been studied across a wide range of mobile animals. For example, schooling in fish has been shown to increase foraging and decrease predation. Our study investigated whether foraging rates of *Acanthus coeruleus*, the blue tang surgeonfish, increased with school size, and whether the benefit was greater when tang foraged on damselfish territories. We observed foraging rates of blue tang in various school sizes in the presence and absence of damselfish territories. We found that individual tang foraging rates decreased as school size increased. Tang foraging rates and school sizes did not differ for tang foraging on or off damselfish territories. Our results suggest that overwhelming territorial damselfish does not provide foraging advantages to schooling tang. Alternatively, schooling behavior could be a tradeoff where predation on individuals is reduced in schools but there is a cost of lower foraging rates per individual. Schooling in tang could also increase access to higher quality resources via overwhelming competitors or predators, in which case the same or greater nutritional gain despite a lower foraging rate per individual. Fish schooling may therefore be determined by environmental conditions that dictate how advantageous it is for an individual to school at a given moment in time. Understanding which factors influence an individual’s decision to join a school, as well as the interaction between factors, may help to further elucidate the advantages of aggregation across mobile animal species.

Key words: Acanthus coeruleus, foraging, schooling

INTRODUCTION

The advantages of intraspecies grouping behaviors such as flocking, herding, and schooling have been studied across a wide range of mobile animals (Pitcher 1986, Caraco 1989, Rands et al. 2004). Members of groups reap benefits such as predator avoidance and increased foraging efficiency, which are often interrelated (Pitcher 1986, Wolf 1987). Grouping might enhance foraging because individual group members can spend less time watching for predators and more time feeding (Foster 1985, Wolf 1987). Additionally, group formation has been shown to decrease the time it takes to search for foraging patches (Foster 1985). Finally, foraging in groups may allow grazers to overcome the defensive strategies of territorial competitors that can exclude grazers from their territories (Foster 1985). The advantages of group formation are therefore linked to both intra- and interspecific interactions.

Research on school formation in fish has emphasized that the benefit of schooling is influenced by a variety of conditions, such as predation pressure and foraging behaviors (Pitcher 1986). For example, schooling enhances foraging and also reduces predation (Foster 1985, Pitcher 1986). Additionally, the ability of large schools of fish to overwhelm territory holders is particularly important for fish that forage on substrate-associated resources such as algal mats that are patchily distributed and vary in quality (Foster 1985). Therefore, schooling behavior in reef fish may be driven by a combination of factors rather than any single factor (Pitcher 1986).

We investigated how inter- and intraspecific factors relate to foraging school size in the blue tang surgeonfish (*Acanthus coeruleus*), an herbivorous species common in Caribbean coral reefs (Foster 1985). Blue tang forage on epiphytic algae in conspecific or mixed-species schools of one to more than 500 individuals (Foster 1985). Additionally, blue tang feed on the algae in territories defended and cultivated by damselfish (Pomacentridae, Foster 1985), which can occupy a large portion of the reef (Ceccarelli et al. 2005). Blue tang schools on the Caribbean coast of Panama derive increased foraging benefit relative to solitary foragers, potentially because damselfish are less likely to
attack schooling individuals (Foster 1985). We tested whether the aforementioned relationship between schooling, foraging, and overcoming damselfish territoriality occurs in a fringing reef on Little Cayman, Cayman Islands. If schooling enhances foraging benefit, then foraging rates, quantified by number of bites of algae, should increase with school size. However, blue tang foraging in larger schools may gain access to higher quality resources; if so, they may forage at decreased rates with the same nutritional gain. If schooling simultaneously enhances foraging benefits and allows blue tang to exploit damselfish territories, then foraging rate in schooling tang should be higher across all areas of the reef irrespective of damselfish territories.

METHODS
We conducted our study in the back reef zone of the Grape Tree Bay fringing reef near the Little Cayman Research Center, Little Cayman, Cayman Islands, B.W.I. Observations were made in the morning (9:00-11:00) and afternoon (14:30-17:30) on 25-28 February 2013. By snorkeling, we observed foraging blue tang surgeonfish in various sizes of intraspecific schools as we encountered them on the back reef. We recorded foraging rate as the number of bites of algae by a focal fish (haphazardly selected within a school) during 30-second observation periods, for a maximum of three observation periods per school. We noted time of day of each observation as either morning or afternoon and assigned each school of fish a unique code so that each schooling group could be included as a factor in the analysis and control for variation due to different groups rather than include this in the unknown error variance. To investigate the effects of damselfish territoriality, we also recorded the number of damselfish territories present within the focal fish’s foraging range per observation period.

Statistical Methods
To test how blue tang foraging rate varied as a function of school size, damselfish presence, and their interaction, we used a linear mixed-effects model with damselfish presence and school size as fixed effects, and schooling group and time of day as random effects. The analysis was performed using JMP 10.0 and the data met all assumptions for the model.

RESULTS
We observed 45 focal fish in schools, and schools ranged in size from 1-18 individuals. There was a total of 67 observation periods because we followed each focal fish for 1-3 trials. Blue tang foraging rate decreased as school size increased ($F_{1,36.86} = 5.83, P = 0.02$, Fig. 1).

Time of day (morning or afternoon) accounted for 31% of the variance in number of bites of algae taken by focal tang, while the schooling group accounted for 23% of variance in number of bites.

The presence of damselfish did not have an effect on foraging rate ($F_{1,63.07} = 1.75, P = 0.19$, Fig. 2), and we found no interaction between school size and damselfish presence ($F_{1,34.7} = 0.017, P = 0.90$).

DISCUSSION
Our results did not support the hypothesis that schooling increases foraging benefit in the presence of territorial fish. Contrary to previous research on other species of tropical fish (Pitcher 1986, Wolf 1987), foraging rate for blue tang
appears to decrease with schooling behavior. We found that damselfish presence did not affect the foraging rate of blue tang regardless of school size at Little Cayman; therefore, the conclusions drawn by Foster (1985) for Panama reefs do not seem to apply to all blue tang populations or other reefs. Previous research has found that large schools of blue tang are less likely than individuals to be attacked by other fish in general, but just as likely to be attacked by damselfish alone (Morgan and Kramer 2004). Therefore, interactions with damselfish alone appear to have no effect on blue tang schooling.

Some of the variation in our data can also be explained by the frequency in which blue tang were observed in differently-sized schools. We collected more data on individuals and small schools than large schools, which resulted in higher variation in the data for small schools. Temporal variation in feeding behavior may have biased our data towards few observations of large schools, as time of day accounted for 31% of the variation in number of bites taken. Tang are found more often as individuals and in smaller schools in the morning and afternoon, and are frequently found in larger schools around noon (Morgan and Kramer 2005).

Additionally, diurnal reef fish have been shown to have higher foraging activity in the early afternoon than in the morning or late afternoon, when the risk of predation is greater (Klumpp and Polunin 1989). We found that the majority of tang were foraging individually in the morning and afternoon, so future studies should include observations of tang schooling in the middle of the day.

Further, tang behavioral mode may explain the higher variability and greater number of observation of small-sized schools. Morgan and Kramer (2005) divided tang behavioral modes into three categories (wandering, territorial, schooling), and evaluated the prevalence of each behavior throughout the day. Density of territorial fish did not change throughout the day, but non-territorial fish that wandered in the morning formed schools at noon (Morgan and Kramer 2005), correlating with the peak foraging time found by Klumpp and Polunin (1989). Based on our observation times, we likely encountered more territorial and wandering individuals than schooling individuals. Additionally, we may have observed territorial tang foraging on their own territories. Future studies on tang foraging should observe each fish for a longer period of time to determine its behavioral mode.

One explanation for decreased foraging rates in schools of blue tang is that schooling behavior may provide a benefit in terms of reduce predation but come at the cost reduced per capita foraging. Larger schools are able to confuse or overwhelm predators, increasing an individual’s chance of survival (Landeau and Terborgh 1986). Smaller surgeonfish also have greater schooling tendencies (Wolf 1987), and smaller reef fish are more vulnerable to predation (Mumby et al. 2006). Future studies should consider exploring the relative contributions and possible trade-offs of predation and foraging to school size.

Additionally, blue tang foraging rates may decrease in schools because schooling might increase access to higher quality resources and therefore allow the same nutritional gain at lower foraging rates. Previous research has shown that grazing herbivores select for resource patches that offer optimal benefits, and suggests that preference for higher quality...
resources could drive aggregation behavior (Wilmshurst et al. 1994). Therefore, the negative relationship we found between schooling and foraging rate may result from the schooling fish’s ability to forage on higher quality resources. Future studies should investigate if resource quality varies with foraging school size by identifying the specific algal species and amount ingested by tang.

Finally, the advantages of schooling in blue tang may be more dynamic and context-dependent than previously thought. Schools are social groups of fish that choose to aggregate, so individuals must constantly reevaluate the costs and benefits of school formation (Pitcher 1986). As discussed by Pitcher (1986), the interacting factors influencing the assembly of fish in schools might vary on a second-to-second time scale rather than remaining constant over a long time scale.

Our study suggests that the advantages of schooling may be more complex than predicted. Other benefits such as access to high quality resources and protection from predators may partially drive school formation in fish. Schooling may therefore be influenced by factors of varying levels of importance that dictate whether it is advantageous for an individual to join a school (Pitcher 1986). Groups of animals are dynamic entities, and understanding the components of group benefits may help further elucidate the advantages of flocking, herding, and schooling across animal species.

ACKNOWLEDGEMENTS
We would like to thank the staff of Little Cayman Research Center (CCMI) for sustenance and natural history knowledge. We would also like to thank Ramsa Chaves-Ulloa, Zachariah Gezon and Brad Taylor for their assistance and feedback.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
A HUMAN-INDUCED TROPHIC CASCADE: EFFECTS OF CONCH HARVESTING ON MARINE PLANTS

AMELIA ANTRIM, GILLIAN BRITTON, COLLEEN COWDERY, VICKY STEIN, ELLEN IRWIN, ELIZA HUNTINGTON

Faculty Advisor: Brad Taylor

Abstract: Trophic cascades dramatically alter the composition and structure of communities. Humans can induce trophic cascades by acting as top predators through activities such as hunting and fishing. In the Caribbean, queen conch (Strombus gigas) populations are in severe decline due to overharvesting. In the South Hole Sound of Little Cayman Island, there is an area open to conch harvesting and adjacent area protected from conch harvesting to allow re-growth of the conch population, providing a natural experiment to test the effect of human conch harvesting on marine plants. We assessed the effects of conch harvesting by comparing nearshore marine plants between the protected and unprotected areas of the sound. We found lower conch abundance and biomass per unit area in the unprotected area than in the protected area. Consistent with the trophic cascade model, we also found increased plant cover and lower macroalgal species richness in the unprotected area, but no significant difference in microalgal biomass between areas of the sound. Our results suggest a trophic cascade in which human harvesting reduces conch populations, thereby releasing marine plants from conch grazing. This human-induced cascade may indirectly affect habitat structure and economic value of the seagrass ecosystem and, perhaps, the adjacent coral reef ecosystem.

Key words: Algae, Strombus gigas, Thalassia testudinum, trophic cascades

INTRODUCTION

Trophic cascades can dramatically alter the composition and structure of communities (Coleman and Williams 2002). Changes in the population of one predatory species have been shown to affect both species abundance and richness indirectly at lower trophic levels (Paine 1966). Humans can cause trophic cascades by functioning as top predators through activities like fishing (Sala et al. 1998, Pace et al. 1999, Jackson 2008). In many cases, these anthropogenic influences can have negative effects on ecosystem balance by changing relative population sizes of interacting species (Pace et al. 1999, Sanchirico and Wilen 2001).

Marine reserves have been established to counteract anthropogenic effects on harvested populations (Sanchirico and Wilen 2001). Marine protection areas (MPA) help manage commercial fish populations and aim to protect marine biodiversity from the effects of overharvesting. By providing refuges for harvested species, reserves may also have indirect effects on the community, as protected species interact with other populations through competition and/or predation (Mumby et al. 2007).

Queen conch (Strombus gigas), a primarily herbivorous marine gastropod, is one example of a species threatened by overharvesting. In the Caribbean, the queen conch has been harvested for centuries and is currently the second most commercially important species (Tewfik and Bene 2000). Expanded export markets and subsequent increases in harvesting pose a risk to these animals (Tewfik and Bene 2000). Conservationists are seeking to preserve sustainable populations of the queen conch by implementing replenishment zones and harvesting limits (Cayman Department of Environment 2010).

In the South Hole Sound off the southern coast of Little Cayman Island, queen conch are harvested by local residents, tourists, and fishermen. In 2007, a law was enacted to help restore the conch population by protecting a portion of the sound from conch harvesting and restricting harvesting in the unprotected area to November through April (Cayman Department of Environment 2010). The creation of an MPA where conch harvesting is prohibited provides an opportunity to test the effects of human conch harvesting on lower trophic levels.

We tested how human removal of conch affects plant richness and density. The top-down
model of trophic cascades predicts that harvesting should result in fewer conchs in the unprotected areas, thereby releasing plants from top-down regulation by their herbivores, increasing plant density (Figure 1). In the absence of predator or herbivore control on lower trophic levels, competitively dominant species are likely to monopolize the population, lowering species diversity (Paine 1966). Thus, we expected to find fewer conch, greater marine plant density and lower plant richness in the unprotected area than in the protected area of the Sound. Alternatively, human harvesting might have little to no effect on conch populations due to high rates of migration from the protected to unprotected area, or conch might have little to no effect on plants because of rapid plant growth rate or plant defenses. In these cases, we would find no significant difference between the conch and marine plant populations of the two areas, indicating that humans are not causing a trophic cascade by harvesting conch.

METHODS
We compared conch and plant abundance in a protected versus unprotected area of the South Hole Sound of Little Cayman Island, Cayman Islands. We established 18, 50 m transects perpendicular to shore (nine in each area), beginning at a depth of approximately 1.1 m. We counted the number of live conch within two meters of each transect. We measured the length of each conch found, then estimated conch biomass using the equation: \[ \log_{10}(\text{wet weight}) = 3.403 \log_{10}(\text{shell length}) - 5.569 \] (Stoner and Lally 1994).

In four transects within each area, we sampled marine plant cover using a 0.25 m² quadrat placed to the left and the right of the transect line every 5 m. We estimated percent cover of marine plants within each quadrant, and identified individuals to species using Marine plants of the Caribbean: a field guide from Florida to Brazil (Littler et al. 1989). At 10-m intervals along the transects we collected microalgal samples by taking sand cores using 118 mL urine cups. We filtered 5 mL from each core onto a 1 mm glass fiber filter. Microalgae collected on the filter was extracted in 4 mL ethanol in the dark for 12 hours and fluorescence of chlorophyll \(a\), an indicator of microalgal biomass, was measured on Turner Designs AquaFluor fluorometer.

Statistical Analysis
To test for differences in conch abundance, percent plant cover, and microalgal biomass between the protected and unprotected areas, we performed unequal variance t-tests rather than pooled variance t-tests because the variances were unequal between the two areas. Because plant cover can decrease with water depth, we adjusted for depth effects in our analysis by performing a pooled t-test on the residuals from a regression of plant cover versus water depth. Upon recognizing that turtle grass comprised the large majority of plants in both areas, we compared turtle grass cover across areas using an unequal variance t-test. To compare the richness of plants across the two areas, we performed an unequal variance t-test on the number of marine plant species observed per transect. Although differences in number of individuals can influence estimates of species richness (Gotelli and Colwell 2001), we did not use rarefaction because the area with greater plant density (unprotected area) had lower richness, an effect that could not be explained by the differences in density. We conducted all tests using JMP 10.0 statistical software, and assumptions for all tests were met.
Figure 2. *Strombus gigas* were more abundant in the area protected from human harvesting compared to the unprotected area of the South Hole Sound, Little Cayman Island.

**RESULTS**

We found seven times more conch per transect in the protected area (mean = 3.33, SE = 0.78) than in the unprotected area (mean = 0.44, SE = 0.24; \( t_{9.5} = 3.53, P = 0.006 \); Fig. 2). Conch biomass per transect was also nearly ten times greater in the protected area (mean protected = 477 g, mean unprotected = 49.5 g, \( t_{9.4} = 3.01, P = 0.01 \)).

We found greater percent cover of marine plants in the unprotected versus protected area of the sound (\( t_{74.5} = 4.35, P < 0.0001 \), Fig. 3), with a mean of 70 % plant cover in the unprotected area and 39 % cover in the protected area. (SE protected = 4.72%, SE unprotected = 5.06%). This nearly two-fold difference was still significant after adjusting for the effects of water depth using the analysis of residuals (\( t_{77.6} = 3.30, P = 0.002 \)).

Percent turtle grass cover was also two-fold higher in the unprotected area, with 69% mean turtle grass cover in the unprotected area and 33% turtle grass cover in the protected area (\( t_{77.5} = 3.53, P = 0.001 \)), irrespective of water depth (\( t_{77.5} = 3.35, P = 0.001 \)). We found no difference in the mean microalgal biomass between areas (\( t_{26.8} = 0.64, P = 0.11 \)). Number of marine plant species per transect was slightly higher in the protected area, with a mean of 2 species per transect in the unprotected area and 3.25 species per transect in the protected area (\( t_{5} = 2.61, P = 0.05 \)).

**DISCUSSION**

We found greater nearshore conch abundance and biomass in the area protected from human conch harvesting compared to the unprotected area, demonstrating that restricting harvesting has indeed had a positive effect on conch abundance, and that human harvesting has had a negative effect. We also found increased plant cover and decreased plant species richness in the unprotected area, but did not find any significant difference in microalgal biomass between the two areas of the sound. Our results suggest a trophic cascade in which human predation on conch lowers conch population size, releasing plants from grazing. This potentially allows
certain dominant species to outcompete other marine plants, resulting in lower species diversity in overharvested areas.

In both the protected and unprotected areas, plant cover was dominated by turtle grass (*T. testudinum*). As queen conch feed on turtle grass (Antczak and Mackowiak de Antczak 2005), conch may be reducing turtle grass abundance in the protected area through grazing. Although macroalgae covered less area than turtle grass in each area, we found decreased macroalgal species richness in the unprotected area compared to the protected area, suggesting that conch consumption of turtle grass enables macroalgae to grow in what would otherwise be dense turtle grass beds. Davis and Fourqurean (2001) found that the presence of turtle grass lowers both growth rate and thalli size in macroalgae through competition for nitrogen. The same study found the reciprocal competitive effects of macroalgae on turtle grass to be significantly lower. Previous studies have shown how predator populations can reduce the abundance of a competitively dominant species, allowing other species to persist (Paine 1966). Conchs may be beneficial to macroalgal species richness because they graze on turtle grass, decreasing competition for nutrients.

While there was a clear difference in macroalgae cover between the areas, there was no difference in microalgal biomass. The similarity in microalgal biomass between areas suggests that conchs may preferentially feed on other plants (e.g. turtle grass or epiphytic algae) over benthic microalgae, or that conch populations are simply not large enough to affect microalgal populations.

Increasing plant species richness may increase habitat heterogeneity of seagrass beds, which can indirectly benefit other ecosystems, such as the adjacent coral reef. Orth et al. (1984) found that habitat heterogeneity can provide increased foraging opportunities for economically and aesthetically important species, such as juvenile coral reef fish. Thus, conch may play a role in providing habitats for species associated with other ecosystems, namely the coral reef, demonstrating the importance of the spatial scale of protected areas for marine conservation at Little Cayman Island. Increased species richness could be economically important to the island, whose economy is largely dependent on ecotourism and fishing; coral reef ecosystems generate over 3.1 billion US dollars in goods and services per year in the Caribbean overall (Burke et al. 2004). Additionally, from an economic standpoint, an increase in shoreline vegetation due to lower numbers of conch could have implications for island residents. Numerous beachfront properties are located along the sound, and increased turtle grass beds outside the protected area may be less desirable for property owners because water clarity is often related to property value (Gibbs et al. 2002). *Viatrix globulifera*, a stinging anemone common in turtle grass beds, may make turtle grass even less desirable for property owners.

Our findings suggest that conch harvesting results in a trophic cascade affecting nearshore marine plants. Understanding the effects of human activities such as fishing and hunting on both target species and non-target species can have important implications for maintaining diverse ecosystems. Trophic cascades are an example of how conservation efforts directed at a single species can have indirect effects on other species’ populations, demonstrating the importance of protected zones for maintaining biodiversity within ecosystems.

ACKNOWLEDGEMENTS
We thank the staff and crew of the Central Caribbean Marine Institute, especially Perry Oftedahl, for all of their support, and Z. Gezon, B. Taylor, and R. Chaves-Ulloa for their guidance and assistance in manuscript review.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED


FISH PREFERENTIALLY ATTACK ALLELOPATHIC ALGAE OVER NON-ALLELOPATHIC ALGAE ON THE CORALS Acropora palmata AND Diploria strigosa

TYLER E. BILLIPP, SETH A. BROWN, JIMENA A. DIAZ, KALI M. PRUSS, AND ELISABETH R. SEYFERTH

Faculty Editor: Brad Taylor

Abstract: Mutualisms between corals and other organisms that contribute to coral’s competitive success are important to understand because of the increasing threats to coral reefs worldwide. Marine algae compete with corals for space, and some of these algae use allelopathic secondary metabolites to bleach and overgrow corals. We explored a potential mutualism between corals and fish that remove competitive algae. We predicted that if fish and coral engage in a mutualism, fish would exhibit a stronger response to allelopathic algae than to non-allelopathic algae on corals. We placed allelopathic and non-allelopathic algae on Acropora palmata (elkhorn coral) and Diploria strigosa (symmetrical brain coral) and found that more fish were recruited to algae placed on brain coral than on elkhorn coral. We also discovered that fish were more likely to bite allelopathic algae than non-allelopathic algae regardless of coral type, suggesting that fish consumption or removal of overgrowing algae was greater for allelopathic algae. Our results suggest a potential mutualism in which fish, sheltered by coral, prevent algal overgrowth on their host coral.

Key words: Acropora palmata, allelopathy, Diploria strigosa, elkhorn coral, Galaxaura oblongata, Halimeda incrassata, mutualism, symmetrical brain coral

INTRODUCTION

In contrast to competition and predation, mutualisms involve two or more species reciprocally providing services such that the costs are lower than the benefits for both parties. Benefits of mutualisms may include nutrition, energy, protection, or transport, among others (Boucher et al. 1982). Previous studies have demonstrated various mutualisms among coral reef species, between corals and zooxanthellae, fish and sea anemones, and fish and cleaner fish or shrimp (Knowlton et al. 2003). Understanding interspecific interactions between corals and their surrounding species is important to reef conservation efforts given that recent research has documented widespread declines in coral populations (Pandolfi et al. 2003) and a shift in reef ecosystems from coral-dominated to algal-dominated (Done 1992).

Dixson and Hay (2012) reported a mutualism between staghorn coral (Acropora nasuta) and two species of gobies (Gobiodon histrio and Paragobiodon echinocephalus) in which the fish, which are sheltered by A. nasuta, removed an allelopathic seaweed (Chlorodesmis fastigiata) that competes with coral. By extracting secondary compounds from the algae and putting them in contact with coral, they established that gobies responded to a chemical cue emitted by the coral. Mutualisms involving the exchange of food and shelter for removal of a competitor have been found in terrestrial systems such as ants and acacia trees, but no previous example of a species chemically cueing consumers to remove its competitors has been found (Dixson and Hay 2012).

Since the mutualism between coral and fish that remove competitive algae had only been documented for A. nasuta in Fiji, we explored the generality of coral-fish mutualisms on a reef in the western Caribbean. We predicted a mutualism would most likely occur with coral of the same genus (Acropora) as that studied by Dixson and Hay (2012). We also tested for the presence of a similar mutualism in another common hard coral (Diploria strigosa). Furthermore, we investigated whether fish behavior changed based on algae type. Rasher and Hay (2010) demonstrated that seaweeds damage corals using lipid-soluble allelochemicals transferred via contact. Therefore, allelopathic algae would pose more
of a threat to coral; if a mutualism is present, fish should have an increased response to algae containing allelopathic secondary metabolites than those without these compounds.

**METHODS**

We performed this research between February 25-28 in Grape Tree Bay, at the Central Caribbean Marine Institute’s Little Cayman Research Center, Little Cayman, Cayman Islands. Our focal organisms were two species of coral, *Acropora palmata* (elkhorn coral) and *Diploria strigosa* (symmetrical brain coral) and two species of algae, *Halimeda incrassata* and *Galaxaura oblongata*. *A. palmata* was chosen because mutualisms have been observed previously between *Acropora nasuta* and gobies (Dixson et al. 2012). *D. strigosa* was chosen for its wide distribution and local abundance in Grape Tree Bay and because it provides important habitat for fish (Buchheim and Hixon 1992). For algae, *H. incrassata* was chosen because it possesses allelopathic secondary metabolites while *G. oblongata* does not possess only damage coral through direct contact. We used clumps of algae to simulate algal overgrowth of coral rather than colonization by settling algae. After introducing an algal clump to the coral head, we observed fish response for 2.5 minutes every 10 minutes for 40 minutes. The first observation

<table>
<thead>
<tr>
<th>Coral type</th>
<th>Algal species</th>
<th>Mean Recruitment ± 1 SE</th>
<th>Mean Approaches ± 1 SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. strigosa</em></td>
<td><em>H. incrassata</em></td>
<td>1.35 ± 0.10</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td><em>G. oblongata</em></td>
<td><em>H. incrassata</em></td>
<td>1.30 ± 0.11</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td><em>A. palmata</em></td>
<td><em>H. incrassata</em></td>
<td>0.74 ± 0.13</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td><em>G. oblongata</em></td>
<td><em>H. incrassata</em></td>
<td>0.52 ± 0.16</td>
<td>0.05 ± 0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>&lt;25 cm from algae</th>
<th>Approaches</th>
<th>Bites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluehead (juv.)</td>
<td>63.6</td>
<td>71.2</td>
<td>51.3</td>
</tr>
<tr>
<td>Dusky Damselfish</td>
<td>14.8</td>
<td>18</td>
<td>30.8</td>
</tr>
<tr>
<td>Saddled Blenny</td>
<td>9.7</td>
<td>0.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Yellowtail Damselfish (juv.)</td>
<td>4.5</td>
<td>4.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Cocoa Damselfish (juv.)</td>
<td>4</td>
<td>4.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Beaugregory (juv.)</td>
<td>0.6</td>
<td>0.9</td>
<td>2.6</td>
</tr>
</tbody>
</table>
interval began when the algae were placed on the coral head. For each observation interval we recorded the number and species of individuals that swam within 25 cm of the algae (recruitment), came within 5 cm of the algae and inspected it (approaches), and bit the algae. Recruitment and approaches may provide evidence for an assessment of threat level posed by introduced algae, while biting shows a direct reaction to algae through removal and/or consumption of them. Each behavior represents an escalation of fish response toward algae. We conducted nine allelopathic and nine non-allelopathic algal trials on brain coral and eight allelopathic and eight non-allelopathic algal trials on elkhorn coral using a different individual coral head for each trial.

To control for possible effects of the lead fishing weights, we conducted trials in which we placed three split-shot fishing weights connected by a small piece of fishing line on a coral head and observed fish response using the same methods. To determine if fish were responding to tactile or visual stimulation by the algae (rather than chemical cues released by the algae or coral), we created algal mimics by cutting clear Ziploc® plastic bags into many strips, bunching them together, and attaching three split-shot fishing weights. We conducted three control trials with weights alone and three algal mimic trials on each coral species.

Statistical Analysis
We used two-way ANOVA to test for differences in fish recruitment (total number of fish within 25 cm of the algae) to each macroalgal species on each coral species. We square-root transformed fish recruitment to meet the assumptions of the analysis.

We tested for differences in the number of approaches by fish to the algae species on each coral species using two-way ANOVA. To standardize the number of approaches for differences in fish abundance between the coral species, we divided the number of approaches by number of fish recruited to each coral species. We adjusted the number of approaches and recruitment for the effects of physical contact and the weights by subtracting the average approaches to plastic algal mimics from the average number of approaches and recruitment. We natural log transformed approaches to meet the assumptions of the analysis.

To test for differences in fish biting algae between coral and algae species we used chi-square tests. All assumptions were met and JMP 10.0 was used for all analyses.

RESULTS
The number of fish present within 25 cm of a focal coral head before experimental manipulation was not significantly different between coral types (F\(_{4,36} = 2.70, P = 0.68\)). After adding macroalgae to corals, fish recruitment was higher to brain coral than elkhorn coral (F\(_{1,3} = 25.52, P < 0.0001\), Fig. 1, Table 1), regardless of the algae species (F\(_{1,3} = 1.31, P = 0.28\)).

After accounting for total fish abundance and for fish attraction to plastic algal mimics, the number of approaches was higher towards allelopathic algae than non-allelopathic but only on brain coral (F\(_{1,3} = 3.07, P = 0.04\), Fig. 2, Table 2). Fish approaches overall were higher for brain coral than elkhorn coral (F\(_{1,3} = 11.44, P = 0.002\)).

Fish bit allelopathic algae more than non-allelopathic algae (\(\chi^2 = 5.67, P = 0.02\), Fig. 3)
regardless of coral species ($\chi^2 = 0.045, P = 0.83$).

Nine fish species were observed within 25 cm of the algae; the majority of observations were of juvenile bluehead wrasse (64%) and of dusky damselfish (15%) (Table 2). Juvenile bluehead wrasse made 71% of the approaches to algae, dusky damselfish made 18%, and the remaining 11% of approaches were made by saddled blennies and juveniles of three other species of damselfish. Juvenile bluehead wrasse also made the majority of bites (51%) followed by dusky damselfish (31%).

**DISCUSSION**

Overall, fish were recruited equally to the two species of algae; however, more fish were recruited to brain coral than elkhorn coral after algae were experimentally added. The increased fish presence around brain coral may have been due to its distribution within the reef system. Brain coral was usually a part of the larger, more spatially complex back reef structure with several other species of coral, whereas elkhorn coral was found on the reef crest, isolated from other coral heads. Normally, elkhorn coral heads grow to a large size and in large interconnected colonies across the reef crest; however local populations have declined dramatically due to white pox disease (Patterson et al. 2002), hurricanes (Wilkinson and Souter 2008), and bleaching (Brown 1997) throughout the Caribbean. Widespread death of elkhorn coral has disturbed habitat structure and connectivity on the reef crest, reducing resources and refugia, and likely reducing abundance of resident fish at the elkhorn coral heads we studied.

The number of fish approaching allelopathic algae was 56% greater than the number approaching non-allelopathic algae on brain coral; however, there was no difference in approaches to the algal species on elkhorn coral. Fish approaches to algae may serve as a preliminary assessment of the threat level. Since Dixson and Hay (2012) found that gobies around *Acropora nasuta* were attracted by chemical cues released by the coral in contact with allelopathic coral, it is possible that the difference in approaches per fish by coral may be due to a larger release of chemicals by brain coral than by elkhorn coral when in contact with *H. incrassata*. Besides potential differences in biochemical pathways between the two corals, the local release of a chemical signal to fish may be limited to healthy coral, since we observed that many of the elkhorn coral heads were partially overgrown with algae or partially dead while brain coral heads tended to be free of algae and appeared healthy. If this is the case, factors that damage corals, such as rising ocean temperatures, lowered pH, and coral disease, may weaken the ability of corals to attract fish that may prevent overgrowth of allelopathic algae. These possible impacts on mutualisms warrant further study. Another possible explanation is that elkhorn coral does not exude the chemical cue documented by Dixson and Hay (2012) in *A. nasuta*, or its density was too low to provide sufficient refuges for its mutualistic fishes. However, for the brain coral, the relatively greater number of approaches to allelopathic algae suggest a possible coral-induced attraction mechanism of the fish to algae that is more damaging to the coral.

Biting of macroalgae by fish was more likely when allelopathic *H. incrassata* was introduced versus non-allelopathic *G. oblongata* regardless of coral species. *H. incrassata* and *G. oblongata* are similar in that both are upright
calcareaous macroalgae found near brain and elkhorn coral. However, *H. incrassata* is an allelopathic green alga while *G. oblongata* is a non-allelopathic red alga. Differences in allelopathy and color are thus the two competing explanations for the increased attraction of fish to *H. incrassata*. While color-based food preferences in fish have been documented in certain salmonids, the color preferences were largely determined by the contrasts between the colors of the food and the background (Ginetz and Larkins 1973). Our study sites were shallow and had particularly clear water and thus both red and green colors appeared to stand out, and no one alga appeared to blend into the reef background more than the other. It is therefore unlikely that the differential fish responses to the algae were caused by algal color, suggesting that the presence of allelopathic chemicals drove fish response. By biting allelopathic algae, reef fish may have provided competitive benefits to the coral against the overgrowth of algae that were potentially more damaging. Therefore, our fish biting results provide possible support for a mutualism between the reef fish we observed and brain and elkhorn coral.

Further study could eliminate color as an alternative explanation for the differences found in approaches and biting by testing fish response to red allelopathic and green non-allelopathic algae. Studies could also test whether chemical cues are the mechanism by which fish respond to these macroalgae by directly applying their chemical extracts to coral, since algal allelopathic chemicals are transmitted exclusively through direct contact (Rasher and Hay 2010). Research could also investigate how fish respond to algae of differing toxicity to coral by testing algae with varied allelopathic capabilities. For example, *Dictyota divaricata* is strongly allelopathic and a known colonizer of coral that might be perceived as a greater threat than *H. incrassata*, which does not typically establish itself directly on heads of coral. Alternatively, *Thalassa testudinum*, or turtle grass, which is not allelopathic and rarely co-occurs with either *D. strigosa* or *A. palmata*, might present even less of a threat than *G. oblongata*, which was observed growing around both types of coral.

Our study provides evidence for a mutualism between brain coral and its resident fish and presents initial support for a mutualism between elkhorn coral and resident fish. Fish may preferentially remove algae that are more detrimental to the coral that fish use for shelter and protection. Understanding mutualisms that increase the competitive ability of coral against algae has implications for coral reef conservation efforts given the recent shift from coral-dominated to algal-dominated reef systems (Done 1992). The competitive success of coral species directly affects the diversity and productivity of reef ecosystems. Conservation of coral reefs is especially important, not only for human aesthetic and economic interests, but to the functioning of global marine ecosystems.

**ACKNOWLEDGEMENTS**

We would like to thank the volunteers and staff of CCMI for their help and support, as well as Brad Taylor, Ramsa Chavez-Ulloa, and Zak Gezon for their insightful feedback on our manuscript.

**LITERATURE CITED**


**SEEKING SANCTUARY: EMPTY CONCH SHELLS AS REFUGIA IN HABITATS OF VARYING STRUCTURAL COMPLEXITY**

**EMILIA H. HULL, ELLEN R. IRWIN, KALI M. PRUSS**

Faculty Editor: Brad Taylor

*Abstract:* Organisms employ a variety of defense mechanisms, such as hiding in refugia, to escape predation. In marine ecosystems, empty shells, such as those of the queen conch (*Strombus gigas*), provide important refugia to many small-bodied organisms such as juvenile fish. Previous research on conch shell utilization has focused specifically on fish, and has not taken into account other taxa that also might be important colonizers of such refugia. To understand how species abundance, composition, and competition for refugia varies across sites with different structural complexity, we surveyed organisms in empty conch shells in seagrass, sand, and coral habitats. We also introduced empty conch shells into coral and seagrass habitats to investigate how colonization of refugia varies among habitats and how species composition in newly introduced shells compares to older shells. Both species richness and total organism abundance were higher in existing shells found in seagrass than in either sand or coral habitats. Furthermore, species composition was similar between coral and sand, while seagrass was less similar to the other two. We found no significant differences in number of individuals or species richness in the introduced shells among habitats, and as few were colonized, we did not quantitatively compare them to the existing shells. Taken together, other factors may be important in determining the role of refugia in various marine habitats besides the increase in structural complexity of added refugia. Our study shows that refuge use varies between different habitats. Understanding how refugia influence species populations can be both economically and ecologically important.

**Key words:** conch shells, recruitment, refugia, *Strombus gigas*

**INTRODUCTION**

Prey employ a variety of defense mechanisms, such as hiding in refugia, to escape predation (Hixon 1991, Scharf et al. 2006). Refugia can limit the extent of predation on a population, and thus may affect abundance and distribution of prey (Schulman 1985). As such, competition for refugia among prey can be intense. Competitive exclusion among prey species in refugia may therefore influence patterns of prey mortality, altering overall community composition (Schulman 1985).

In marine ecosystems, empty shells provide important refugia for many marine animals, particularly small-bodied organisms and juvenile fish species, which are highly susceptible to predation (McLean 1983, Wilson et al. 2005). Empty shells may be particularly crucial for coral reef fish, whose recruitment and early survivorship can be related to the availability of refugia (Schulman 1985, Hixon 1991, Lingo and Szedlmayer 2006). In addition, shells can increase the amount of refugia in habitats of low structural complexity, which provide few places for prey to hide (Scharf et al. 2006). Therefore, use of shells in areas of low structural complexity may be high, as refugia are a limiting resource (Almany 2004). Shells can thus influence species composition in ecosystems by altering prey competition and predation (McLean 1983, Wilson et al. 2005). For example, adding scallop shells to sandy and rocky sea bottoms has been found to increase invertebrate species diversity (Guay and Himmelman 2004).

In the Caribbean, millions of queen conch (*Strombus gigas*) are harvested annually and their shells discarded randomly, sometimes back into the marine environment where many organisms inhabit them to avoid predators (Wilson et al. 2005). The discarded shells become important refugia in habitats of low structural complexity, such as sand or hard pan areas, potentially providing habitat for fish and other organisms in those areas (Wilson et al. 2005). Previous research on conch shell utilization has focused specifically on fish (Wilson et al. 2005), and has not taken into account other taxa that also might be important colonizers of such refugia. In addition, competition for conchs among different taxa has...
not been explored, particularly in habitats of varying structural complexity.
To understand how species abundance, composition, and competition for refugia varies across sites with different structural complexity, we surveyed organisms in empty conch shells in sand, seagrass, and coral habitats (representing low, medium and high structural complexity, respectively). Since refugia are more valuable in areas of low structural complexity (Wilson et al. 2005), there should be an increase in number of individuals and species richness in refugia in habitats of decreasing structural complexity. Alternatively, fewer available refugia could result in greater competition in areas of low structural complexity, resulting in a decrease in the number of individuals and richness in refugia. In addition, we investigated how colonization of refugia varies between habitat types and how species composition differs between newly introduced shells and older shells. As colonization often occurs via successional processes (Connell and Slatyer 1977), communities in old and new refugia should differ as initial colonizers may be excluded later by other species that can outcompete them. Alternatively, there may be only specialized species that inhabit conchs, therefore species composition would be similar between introduced and existing shells.

METHODS
We conducted our study in Preston Bay on Little Cayman, Cayman Islands, B.W.I. on March 4-7, 2013. We surveyed all empty conch shells between the shoreline and fringe reef along a fifty meter stretch and recorded the habitat in which we found shells. We classified conchs found within one meter of the reef as coral habitat. We flushed the contents of each shell into a net to count and identify the organisms inside to lowest taxonomic level possible (typically species). We also classified the organisms we found into feeding guilds (Humann 1997, Humann and Deloach 2002, Colin 1988) to determine whether the presence of different feeding guilds affected species composition. We used a Nikon Coolpix 5X wide optical zoom underwater camera to take pictures of species we could not identify in the field.

To test colonization, we placed ten empty conchs in seagrass and ten in coral habitat. Due to a limitation in the number of empty conchs available, we did not test colonization in sand (the least common habitat in the bay). We placed shells in the coral habitat within one meter of the reef. All conchs were between 19 and 25 cm long and were placed facing upwards, spaced five meters apart from one another, with a numbered stone adjacent to serve as a marker. We chose to orient the shells upwards because we had seen inhabited conchs oriented in that manner and we thought it would be easier for organisms to colonize them. Four days after introduction, we re-collected the shells and used the same methods described above to survey the contents of the shells.

Statistical Analysis
In both existing and introduced conchs, we tested for differences in species richness (total number of species found per conch) between habitat types using ANOVA followed by Tukey HSD post-hoc comparisons. To test for differences in the likelihood of being colonized among habitat types in both introduced and existing conchs, we used a chi-square analysis. To test whether total abundance (total number of organisms found in each conch) varied by habitat, we used Wilcoxon/Kruskal-Wallis test and the Wilcoxon Method for post-hoc comparisons.

We tested co-occurrence of species as a function of habitat with an EcoSim co-occurrence model (Gotelli and Entsminger 2012). Not enough introduced conchs were colonized to effectively test for co-occurrence, thus we used the co-occurrence model for existing conch only. To test how species composition (the relative abundance of different species groups) differed by habitat in existing conch, we used percent similarity indices. If any species exhibited a strong trend in differing abundance between habitats, we did not group it with any other species. We grouped other species by taxa and similar trends in their abundance by habitat. Unless otherwise stated, we used JMP 10.0 to test assumptions for and run all analyses.
RESULTS
Species richness in existing conchs differed significantly across habitats ($F_{2,120} = 9.69$, $P < 0.002$, Fig. 1). Existing conchs in seagrass (mean ± 1 S.E. = 2.26 ± 0.23) had significantly more species than conchs in sand (1.50 ± 0.19) or coral (1.00 ± 0.17). The total abundance in existing shells also varied significantly between habitat type: conchs in seagrass had a significantly higher number of organisms than sand or coral (chi-square = 19.08, $P < 0.0001$, df = 2). Furthermore, the shells in seagrass were the most likely to be inhabited (95.0%), followed by sand (76.1%) and coral (59.0%; chi-square = 13.67, $P = 0.001$, df = 2, Fig. 2).

The relative abundance of different species groups in existing shells was similar between coral and sand, while the relative abundance of species groups differed between grass and sand and coral and grass (Table 1). The co-occurrence of species (C-score) in conchs was not significantly different from random in seagrass ($P = 0.82$), coral ($P = 0.54$), and sand ($P = 0.23$).

For introduced conchs, species richness and total abundance were not significantly different between seagrass and coral habitats. We found that the likelihood of being colonized in seagrass and coral habitats was not significantly different, with 60% and 40% respectively. We observed that ocean surgeonfish and crabs (hermit and red-ridged clinging) were found most often in the empty conch shells placed in both coral and seagrass habitats.

Fewer introduced conchs were colonized relative to the proportion of older, existing conch in corresponding habitats (coral: 40% vs. 59%; seagrass: 60% vs. 95%). Furthermore, fewer total species were found in introduced shells (six species between both habitats) than in existing shells (more than 25 species). We did not compare the introduced and existing shells further because so few species were found in introduced shells.

DISCUSSION
Use of empty conch shells as refugia, in terms of total abundance and species richness, did not increase as adjacent habitat structural
complexity decreased. In seagrass, a habitat of medium complexity, we found the highest species richness and total abundance per shell. Sand was the least structurally complex of the three habitats but species richness and abundance inside conch was lower than in seagrass. Contrary to our results, previous studies (Wilson et al. 2005) that investigated fish use of conch shells, found that shells in sand were more likely to be inhabited than those in seagrass. However, we observed that there appeared to be fewer total organisms in sandy habitats, indicating that there may have simply been fewer organisms to recruit to refugia in the first place. Our observations align with previous studies (Jenkins and Wheatley 1998), which found lower fish abundance and richness in sandy habitats compared to coral or seagrass habitats.

Of the three habitats, conchs in the coral reef had the lowest species richness and total abundance. Previous studies (Wilson et al. 2005) have also found that shells in coral habitat were less likely to be utilized, and suggested that coral reefs are complex habitats with many refugia; thus, conch shells in reef habitat have less relative value. Moreover, other factors not included in this study could influence refugia use in the different habitats. For example, we did not measure or compare predation pressure between the habitats, which Beukers and Jones (1997) suggest is an important factor, along with habitat complexity, in influencing fish abundance and diversity.

While the conchs in sandy and coral habitats had similar species composition, the species composition of conchs found in seagrass was different from both the coral and sand habitats. The proximity of sandy habitat with coral likely enabled high exchange between the two habitats. Moreover, certain species, worms especially, were found nearly exclusively in seagrass. Previous studies (Stoner 1980) have found that some the biomass of some worm species is directly related to macrophyte abundance, perhaps due to the increased carbon production and organic matter characteristic of seagrass (Orth et al. 2006). Seagrass habitat likely supports a different community than that of coral or sand; however, we found little difference in the proportion of herbivores (which, due to the nature of the habitat, we expected to dominate) in conch in seagrass versus sand or coral (33%, 26% and 40% respectively). Thus, the different species composition found in conch in the seagrass habitat is not driven by a greater number of herbivores.

We found no evidence to suggest that competition structured the communities in conchs in any of the three habitats. In coral habitat, there may be enough refugia that species do not compete for them. In sand and seagrass there is low structural complexity, which could theoretically lead to high competition for valuable refugia. In sand, we observed a low abundance of organisms: thus, there would be low levels of competition between them. There could have still potentially been competition over conchs in seagrass; if the existing conchs were established communities and species had already been competitively excluded one another, we would find no evidence for competition.

Species richness and abundance did not differ by habitat in the newly introduced conches. However, the percentage differences may be biologically important for such a short time period. In the newly colonized shells, the two most commonly found species were juvenile ocean surgeonfish and hermit crabs in both seagrass and coral habitats. Yet few hermit crabs were found in the older, existing shells. Due to time constraints associated with our study, we were not able to explore a long-term successional pattern in colonization. Previous studies have shown that competition for refugia can competitively displace species (Shulman 1985); therefore future studies should explore whether hermit crabs or other organisms are subsequently displaced by competitors.

Our study indicates that there are other, unknown factors that help determine the role of refugia in different marine habitats. Future work could investigate whether greater numbers of empty conch shells protect, or even increase the abundance of particular populations of coral reef organisms. Seagrass beds serve as important nurseries to juvenile fish (Beck et al. 2001); a greater number of available refugia in these habitats could increase fish populations, both in the short and long term. Furthermore, little work has been done to investigate how increased prey
refugia may affect predator populations. Future studies might examine whether a bottom-up effect exists due to refugia, and whether an increase in available refugia may help increase biodiversity on the reef as a whole.

Our study shows how refuge use can vary between different habitats. The availability of suitable refugia has implications for prey populations, and thus the entire marine ecosystem. Understanding the relationship between habitat and refugia use can have economic benefits: refugia addition into specific habitats has been shown to increase populations of economically important species, like the spiny lobster (Clayton et al. 2010). Furthermore, refugia in certain habitats may be crucial to maintaining marine communities, since organisms face not only the threat of predation, but also various negative effects from humans. Ocean acidification, for example, negatively impacts the marine organisms themselves, but also the habitats they rely on for refuge (Hoegh-Guldberg et al. 2007): these effects can vary by habitat, making it more important to know which habitats to add refugia to in order to have the greatest impact. Understanding the importance of refugia is essential to consider in the face of the current dramatic declines in marine habitat.

ACKNOWLEDGEMENTS
We would like to thank the staff of Little Cayman Research Center (CCMI) for sustenance, and Perry Oftedahl for his time and assistance. We would also like to thank Ramsa Chaves-Ulloa, Zachariah Gezon and Brad Taylor for their assistance and feedback.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
Jenkins, G.P., and M.J. Wheatley. 1998. The influence of habitat structure on nearshore fish assemblages in a southern Australian


LET THE WILD RUMBLES BEGIN: DE-ESCALATION OF CONFLICT THROUGH ACOUSTIC AND VISUAL SIGNALS IN MANTIS SHRIMP (*NEOGONODACTYLUS OERSTEDII*)

VICTORIA D. STEIN, COLLEEN P. COWDERY, AND TYLER E. BILLIPP

Faculty Editor: Brad Taylor

Abstract: Territorial disputes between animals can greatly impact an individual’s fitness through access to resources and physical costs incurred in the dispute. Among animals that possess dangerous weapons there is incentive to de-escalate aggression to reduce the cost incurred during uneven conflicts. This de-escalation could be achieved through signals that minimize physical contact. In the case of the mantis shrimp *Neogonodactylus oerstedii* territorial conflicts might be partially resolved through acoustic and visual displays. We observed intraspecific conflicts between same-sex pairs of *N. oerstedii* and recorded visual and acoustic data, and then replayed acoustic signals from those trials to different individual *N. oerstedii* to examine responses to the signals. In observational trials, physical contact in the form of strikes, using their smashing raptorial appendages, decreased as the number of acoustic signals increased and as the difference in body size between the two *N. oerstedii* increased. These results suggest that visual and acoustic signals may serve to prevent physical contact, which can result in damage or even death, particularly for individuals differing in body size. In audio playback trials, smaller *N. oerstedii* were more likely to exhibit a defensive behavior, implying that the acoustic signals might be effective at preventing conflicts. *N. oerstedii* territorial behavior and intraspecific conflict in general could be regulated by de-escalation of unnecessary violence as a response to honest signals.

Key words: conflict de-escalation, honest signals, *Neogonodactylus oerstedii*, territorial behavior

INTRODUCTION

Animals frequently find themselves in conflict over resources such as food, shelter, and reproductive opportunities, which are often tied to territory and territoriality (Burt 1943, Foster 1985, and Mathis 1990). The outcome of a territorial dispute is essential to each individual’s fitness, and as such, disputes could escalate to violence. Among animals that possess dangerous weapons, direct physical conflict could potentially be very costly (or even lethal) to one or both parties.

Intraspecific communication can help de-escalate conflicts. In many such cases, animals signal their fighting ability or resource holding potential (RHP) (Maynard Smith and Parker 1976; Parker and Rubenstein 1981) by performing ritualized displays before or instead of resorting to physical conflict. Each organism, or combatant, can assess its chances of winning the fight and choose to abandon the potential conflict if the odds are not favorable. For example, male red deer signal their RHP by performing a structured progression of side-on displays and bellows that allows them to settle nearly all disputes before they escalate to violence (Clutton-Brock and Albon 1979). Male anoles also use dewlap and pushup displays to settle conflicts before escalation (Forster et al. 2005; Britton et al. 2013). These signals must be honest indicators of the likely outcomes of conflicts before the actual investment and risk in the fight is made (Clutton-Brock and Albon 1979).

One organism hypothesized to have the sensory capability, weaponry, and competitive nature that might lead to a need for such conflict de-escalation behavior is the mantis shrimp, a stomatopod, and a member of the class Malacostraca in the phylum Arthropoda (Rose 2009). Mantis shrimp can use their raptorial appendages to strike at speeds of 20 m/s, with enough force to break fingers and the shells of their prey, which can include snails and mussels along with more soft-bodied animals (Geary et al. 1991, Patek and Caldwell 2005). Mantis shrimp also have ritualized territorial displays at all times of the breeding cycle, indicating that their territoriality is related to food or shelter rather than reproductive space or opportunity (Dingle and Caldwell 1969). These displays can involve head-rearing (Hazlett 1978), meral spreading (Hazlett 1979), chemical cues (Caldwell 1979) and possibly even acoustic signals (Patek and Caldwell 2006). Acoustic signals, known as rumbles, are a recent
discovery and little is known about their use in mantis shrimp communication; however, Patek and Caldwell (2006) have hypothesized that the sound is related to territoriality.

Visual and acoustic signals in mantis shrimp could provide an example of multisensory communication systems utilized for conflict de-escalation by limiting potential physical costs to both parties in a territorial interaction. We hypothesized that mantis shrimp use visual and acoustic displays in order to avoid physical contact. Specifically, we tested how intraspecific interactions and acoustic signal playbacks affected territorial conflicts among Neogonodactylus oerstedii mantis shrimp.

METHODS

Study System

N. oerstedii is a smashing stomatopod that reaches a maximum length of 7 cm (Caldwell 2005). The species is widespread through the Caribbean and the east coast of the Americas from Florida to Panama (Caldwell 2005). N. oerstedii live in sand or coral burrows in and around reefs and seagrass flats, and actively defend their burrows. Our acoustic analysis of preliminary observations confirmed that N. oerstedii produce acoustic signals, or rumbles. Individuals were also observed performing meral spreads, which are used as aggressive displays in other stomatopods (Dingle 1969). We captured and performed trials on 36 N. oerstedii at Little Cayman Research Center on Little Cayman, Cayman Islands, B.W.I. in the western Caribbean Sea from March 4 to 7, 2013. Individuals were collected from coral burrows found in seagrass beds and coral rubble in the littoral zone of Grape Tree Bay and held separately in 120 mL plastic cups to prevent intraspecific aggression. Individuals were measured from tips of eyes to end of telson (tail) spines (Caldwell and Dingle 1979) and sexed by visual inspection of gonopods (McLaughlin 1980). We tested all individuals within 24 hours of collection.

Observational Trials

To observe intraspecific communications between N. oerstedii, we paired 24 individuals into 12 same-sex pairs. We conducted observational trials in a 8 L clear plastic container (Caldwell 1979) filled with 4 L of

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swimming</td>
<td>Use of swimmerets, legs, and body to move through the water column</td>
<td>Neutral</td>
</tr>
<tr>
<td>No Movement</td>
<td>On bottom of container, no motion or display</td>
<td>Neutral</td>
</tr>
<tr>
<td>Displaying</td>
<td>Meral spread display, head and eyes raised, mouthparts visible</td>
<td>Aggressive</td>
</tr>
<tr>
<td>Approaching</td>
<td>Front-facing movement along the bottom of the container toward conspecif</td>
<td>Aggressive</td>
</tr>
<tr>
<td>Striking</td>
<td>Forceful use of raptorial appendages to physically hit conspecific</td>
<td>Aggressive</td>
</tr>
<tr>
<td>Avoiding</td>
<td>Swimming away from the approaching conspecific</td>
<td>Defensive</td>
</tr>
<tr>
<td>Curling Defensively</td>
<td>Presenting a smaller, more-defended target with telson blocking access to underbelly</td>
<td>Defensive</td>
</tr>
<tr>
<td>Clinging to Speaker*</td>
<td>Clinging to digital voice recorder in bag in container</td>
<td>Neutral</td>
</tr>
</tbody>
</table>

Table 1. Definition of terms used to describe and quantify N. oerstedii behavior. * marks behavior seen only in playback trials.
water and outfitted with a hydrophone connected to an Olympus WS-801 digital voice recorder. The hydrophone was hung ~2.5 cm above the center of the container bottom from a rod placed on top of the container. We simultaneously introduced both mantis shrimp into the container and observed and recorded the behavior of each individual for 5 minutes. The behaviors established for *N. oerstedii* were swimming, no movement, displaying, approaching, striking, avoiding, curling, and clinging to the speaker (Table 1). All behaviors were classified either as aggressive, defensive, or neutral (Table 1). We also recorded the audio of each trial. Each mantis shrimp was paired with a different individual twice, for a total of 24 trials.

Trials using the same mantis shrimp were separated by at least one hour to minimize effects of non-independence. We transcribed the recorded audio (rumbles) made by each individual during the trial and combined them with our visual observations of behavior.

**Playback Trials**

We tested the effect of the acoustic signal recorded during observational trials by playing the signal to one mantis shrimp and recording their behavioral and acoustic responses. We cut and looped one rumble (approximately one second) audio file taken from our first communication trial (the signal was generated by one of the two largest females captured in our study, over 3.5 cm in length) to produce a two minute audio playback. The signal was played with the Olympus digital voice recorder, which was enclosed in a waterproof bag and suspended approximately ~2.5 cm under the water surface along one wall of the same container used in the observational trial. To control for unintended effects of using the recorder for playback, we also performed identical trials using two minutes of blank audio. We played both audio files to 10 *N. oerstedii* (5 male, 5 female); play order was chosen randomly by coin toss. We observed and recorded their behavior (Table 1) and recorded the audio for each trial.

**Statistical Methods**

We used multiple regression to examine the factors affecting the number of strikes (physical contact, as in Table 1), with number of rumbles, sex, and ratio of body sizes as explanatory variables. We excluded sex from the final model because it did not describe a significant amount of variation in strikes. We then tested the direct relationship between number of rumbles and body size ratio using simple linear regression.

We also conducted a principal components analysis (PCA) to examine variation in observed behaviors during our playback trials. PC1 was plotted against PC2 to create a scatter plot showing variance in behavioral combinations displayed by each of the mantis shrimp. We analyzed the PC1 and PC2 data by combining data points by sex, treatment, and body size (greater or lesser than the mean body size). A visual analysis of sex showed no trends, so sex was excluded from further testing. We tested whether the variance of these values for large and small shrimp around PC2 was equal using Levene’s test for equal variance.

**RESULTS**

The results of the multiple-regression analysis showed that both number of rumbles and ratio of body sizes negatively affected the number of strikes that occurred in a trial (Fig. 1) and explained 27% of variation in strikes. Ratio of body sizes had a significant effect on the number of strikes (*F*₁,₂₃ = 6.27, *p* = 0.021, Fig. 1A), while number of rumbles displayed a strong trend (*F*₁,₂₃ = 4.18, *p* = 0.054, Fig. 1B).

The number of rumbles generated by both mantis shrimp in a trial was not related to ratio of body sizes (*p* = 0.958, *R*² = 0.00012); pairs with small differences in body size generated

<table>
<thead>
<tr>
<th></th>
<th>PC 1</th>
<th>PC 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Approach</strong></td>
<td>0.57427</td>
<td>-0.12939</td>
</tr>
<tr>
<td><strong>Display</strong></td>
<td>0.43990</td>
<td>0.05943</td>
</tr>
<tr>
<td><strong>Curl</strong></td>
<td>0.57427</td>
<td>-0.12939</td>
</tr>
<tr>
<td><strong>Swimming</strong></td>
<td>0.37385</td>
<td>0.23831</td>
</tr>
<tr>
<td><strong>No Movement</strong></td>
<td>-0.00911</td>
<td>0.81855</td>
</tr>
<tr>
<td><strong>Clinging to Speaker</strong></td>
<td>-0.08402</td>
<td>-0.48597</td>
</tr>
<tr>
<td><strong>Variance Explained</strong></td>
<td><strong>44.25%</strong></td>
<td><strong>19.36%</strong></td>
</tr>
</tbody>
</table>

*Table 2. Principal components analysis showing the first three principal components which together explain over 60% of the variation found in observed behaviors between playback trials.*
Figure 1: The number of strikes decreased with both (A) body size and (B) number of rumbles in mantis shrimp *N. oerstedii*.

roughly the same number of rumbles as pairs with large differences in body size.

Principal components 1 and 2 accounted for a total of 63.6% of the behavioral variation in the playback trials (Table 2). Principal component 1 explained 44.3% of variation, and was most affected by approach, display, and curl behaviors (57.4%, 44.0%, 57.4%, respectively). Principal component 2 explained an additional 19.4% of variation, and was most affected by clinging behavior (81.9%). When PC1 was plotted against PC2, all control trial data were clumped around the origin; whereas, treatment data points were more widely distributed. Dividing the data based on body size, individuals smaller than the mean body size (3.12 cm) were clumped below the x-axis created by PC2. Individuals larger than the mean body size were clumped, but overlapping with the small mantis shrimp.

There was a significant difference in the variance of behaviors between large and small stomatopods (Levene’s test for equal variance: $F_{1,18} = 4.785, P = 0.0421$). We found an almost threefold difference in the standard deviation of behaviors from the x-axis between big and small mantis shrimp (1.43 and 0.51, respectively).

**DISCUSSION**

Our results show that *N. oerstedii* de-escalates conflict through visual and acoustic signals, as evidenced by observations and by analyses of aggressive intraspecific interactions and responses to auditory signal playbacks. In the territorial trials, we found that the number of rumble vocalizations and relative body size ratio between the two mantis shrimp predicted the number of strikes delivered. Examining the influence of rumbles and relative body size on the model separately, body size ratio was the most important factor deciding the level of physical conflict, with rumbling as an important but not quite significant factor. This result suggests that assessment of an individual’s body size and the use of rumbling vocalization serves to lower levels of unnecessary physical conflict in cases where odds are not favorable for one individual (i.e. when the competitors have unequal body sizes and risk is high for the smaller competitor). However, when conspecifics are more equally matched in size, visual and auditory signals do not seem as effective at deterring conflict, perhaps because the competitors have assessed their opponent and the odds of favorable outcome to physical conflict are acceptable (Parker and Rubenstein 1981; Hammerstein and Parker 1982). If this concept is true for mantis shrimp, then body size and rumbling are likely honest signals of physical ability; however, further testing would be needed to determine what exactly the signals indicate.

PCA analysis of playback trials showed that mantis shrimp have a wide array of responses to the recorded rumble vocalization, whereas the reactions shown during the control trial were all very similar to one another and did not show the
same range of behaviors. When further analyzed by body size, the significant difference in variance found between big and small stomatopods was centered around the data from PC2, which was 81.9% determined by clinging behavior. The tight grouping of small stomatopods shows that when exposed to rumbling vocalization recorded from a large stomatopod, small stomatopods are likely to exhibit clinging behavior. We speculate clinging to be a highly defensive behavior as the stomatopods attempted to hide behind the speaker’s bag (the only refuge in the container); this type of avoidance behavior has been shown in various animals (Kelly and Drew 1976, Valdimarsson and Metcalfe 1998, Blanchard and Blanchard 2005). Our results show an increase in striking as body size ratio decreased (Fig. 1), suggesting that highly aggressive behaviors such as physical conflict are greatly influenced by the relative body sizes of the competitors. Furthermore, highly defensive behavior such as seeking refugia in response to rumbling is also greatly influenced by relative body sizes.

We speculate that rumbling is indicative of the body size of the sound producer. Our speculation is supported by the avoidance behaviors (clinging to speaker) observed in small stomatopods in response to rumbles recorded from a large stomatopod. Given the importance of burrowing in mantis shrimp life history (Dingle and Caldwell 1969), it makes sense that a non-visual cue would aid in settling disputes when the prospective competitor is not within direct line of sight. Our study found no discernible trends between sexes in relation to strikes, rumbling, or behavioral responses to rumbles. This lack of differentiation in territorial behavior between sexes may warrant further research. Mantis shrimp have been shown to defend territory equally among sexes, because they have equal investment in those territories and there is likely equal inter- and intra-specific competition (Foote 1990); further tests could focus on investment and defense of resources between male and female mantis shrimp to determine exact causes of this behavior.

![Figure 2: Scatterplot of principal components 1 and 2, showing the distribution of behavioral combinations used by big and small stomatopods when exposed to recorded rumble vocalizations (small = solid circles; big = empty circles).]
The results of this experiment have implications for study of multisensory communication systems in which several forms of signaling occur simultaneously. In any such system, it is possible for the signals to act in concert with each other, reinforcing the message and improving the chances that it be received as intended without alteration or exploitation (Johnston 1995). This appears to be the case in this system, where both visual and auditory signals are used to convey territorial messages, possibly as honest indicators of body size and physical ability. The alternative is that multiple lines of communication may be sending different messages, some of which may be dishonest. If an organism wishes to use a dishonest signal to attempt to deceive a potential mate or competitor, they may actively send a dishonest signal, which is contradicted by an unconsciously sent secondary signal (such as physical appearance). Mantis shrimp are known to “bluff” when newly molted, displaying their meral spots like they typically would despite the fact that their exoskeleton cannot resist blows (Adams and Caldwell 1990). A scenario of contradictory signals may destabilize a system of ritualized behaviors aimed at de-escalating conflict, as de-escalation systems rely on honest assessment of competitors resulting in reliable estimations of risk. However, Rowell et al. (2006) suggest that when broadcasting multiple signals, a combination of honest and dishonest signals may be still be successful without destabilizing. If the “dishonest” signal is viewed to be moderately reliable or unreliable, it may elicit different reactions from different receivers based on the pay-offs those individuals expect to get, thus giving offering a variety of reactions to the signaler. With this model, it is possible that both honesty and dishonesty is present among territorial auditory and visual assessment in mantis shrimp.

Our study provides new data on a system of complex multisensory behaviors which de-escalate violence; future research should examine Stomatopoda behavior for further understanding of the relationship between signal honesty and de-escalation systems, and the effect of multiple lines of communication on the robustness of a communication system.

ACKNOWLEDGEMENTS
We would like to thank Brad Taylor, Ramsa Chaves-Ulloa, and Zachariah Gezon for their advice, assistance, and encouragement in performing these observations and experiments. We would also like to thank the Little Cayman Research Center (CCMI) staff and volunteers for their patience and support.

AUTHOR CONTRIBUTIONS
CPC performed acoustic analyses; TEB and VDS performed observations. Authors contributed equally to other components.

LITERATURE CITED


IMPORTANCE OF HABITAT FRAGMENT SIZE, DISTURBANCE, AND CONNECTIVITY: AN EXPLORATION OF SPECIES DIVERSITY IN TROPICAL TIDAL POOLS

ELISABETH R. SEYFERTH, SAMANTHA C. DOWDELL, MARIA ISABEL REGINA D. FRANCISCO, JIMENA DIAZ, AND GILLIAN A.O. BRITTON

Faculty Editor: Brad Taylor

Abstract: Understanding factors that affect species diversity is increasingly important as we face rapid global losses of species due to anthropogenic changes. Increasingly, individuals, organizations, and governments are seeking ways to conserve biodiversity, often by creating natural reserves protected from the effects of human activities. However, there remains divided opinion as to which factors are most important to consider for maintaining viable populations and species diversity when planning reserves. To gain insight into how best to conserve biodiversity with limited space and resources we looked at the factors driving species evenness and richness in tropical tidal pools. We found that tidal pool height above the ocean was the strongest factor explaining species richness and evenness. We also found that species richness increased with the volume of the tidal pool and tended to increase as salinity decreased. Further, species evenness increased as salinity decreased. Our study suggests that reserves should be constructed to maximize connectedness with a species source (whether that is another reserve or the equivalent of a mainland). While biodiversity conservation efforts should focus resources into connecting nature reserves, preventing severe disturbance and increasing reserve size remain important for maximizing species diversity.

Keywords: Biodiversity, habitat fragmentation, species evenness, species richness, tidal pools

INTRODUCTION
Species diversity is valuable to both ecosystems and human populations. Increasing species diversity within a biological community has been shown to increase its resilience (Walker et al. 1999, Elmqvist et al. 2003). Higher biodiversity may increase the number of functional groups or redundancy within an ecosystem, which increases the chances that an ecosystem will recover from disturbances such as disease or habitat fragmentation (Walker et al. 1999, Elmqvist et al. 2003). Additionally, species diversity is important for human populations as we gain many ecosystem services, such as nutrient cycling and water purification, from diverse ecosystems (Elmqvist et al. 2003). Biodiversity also fuels economic markets such as ecotourism and bioprospecting (Edwards and Abivardi 1998).

However, human-driven habitat fragmentation, introduction of invasive species, over-harvesting, and pollution are driving a global decline in species diversity (Elmqvist et al. 2003). Recognizing the importance of biodiversity, individuals, organizations, and governments are seeking ways to conserve biodiversity, often by creating natural reserves protected from human influence (Metzger 2001). However, there remains divided opinion as to how a reserve should be constructed to best maintain viable populations and species diversity (Simberloff and Abele 1982, Metzger 2001, Uezu et al. 2005). In the current single large or several small reserves (SLOSS) debate, some researchers assert that the spatial extent of a reserve is the most important factor for maximizing species diversity (Diamond 1975, Guirado et al. 2006), while others state that connectivity between reserves may be more advantageous (Simberloff and Abele 1976, 1982, Almany et al. 2009).

To gain insight into how best to conserve biodiversity with limited space and resources, we studied the relative influence of size, connectivity, and disturbance of habitat fragments on species diversity. We tested the factors driving species evenness and richness in tidal pools varying in distance from and height above the ocean, water volume, density of refugia, and risk of desiccation. If fragment size explains diversity, the largest fragments will contain the greatest species diversity as predicted by the theory of island biogeography (MacArthur and Wilson 2001). Thus, tidal pools with the greatest water volume would have the highest species diversity. If fragment connectivity explains diversity, the
fragments with the greatest connectivity to, and least isolation from, the species source will contain the greatest diversity as predicted by island biogeography theory (MacArthur and Wilson 2001). In this case, tidal pools closest to the ocean (i.e. with the highest connectivity to the species source) would have the greatest species richness and evenness. Alternatively, if disturbance is the main factor driving diversity, species diversity will be best explained by the intermediate disturbance hypothesis, which predicts that the greatest diversity will be found in environments experiencing intermediate levels of disturbance (Connell 1978, Sousa 1979). Based on intermediate disturbance hypothesis, pools with an intermediate level of desiccation would have greater species richness and evenness.

METHODS
We conducted our study along the western shore, near the lighthouse, of Little Cayman, Cayman Islands, B.W.I. on March 4-7, 2013. We used a stratified random sample to select tidal pools by haphazardly selecting five pools in each categorical combination of size (small, medium, large) and distance from the ocean (close, medium, far). We randomly selected three pools from each combination for a total of 27 pools. Six pools were completely dry by the time we began to collect species diversity data, and these pools were replaced with pools randomly selected from the same category.

For each pool, we estimated species evenness and richness by counting the number individuals of each species at and below the waterline. For species that were abundant and that visually appeared evenly distributed within a pool, we used a 0.0625 m² quadrat to estimate abundance in each pool. We measured distance of each pool from the ocean with a measuring tape. We also used a level attached to string to measure the relative height of each pool, using a fixed height on the shoreline as a reference. To calculate height above the ocean, we multiplied all our values from distance below the shoreline by negative one and added the height of a pool at sea level to all other values, making the lowest height equal to zero and all other heights positive. Since salinity increases with evaporative water loss, we used the salinity of pools as a proxy for disturbance by desiccation. We measured salinity of each pool with a YSI 63 pH, salinity, conductivity, and temperature meter. In addition, because the number of refugia might affect species diversity, each group member individually estimated relative density of refugia within a pool by scoring each pool on a scale from 0-3 (0 = no refugia, 1 = low density, 2 = medium density, 3 = high density). We averaged our scores for each pool to create a refuge index.

We calculated pool water volume by estimating surface area of the water within a pool by dividing the pool into approximate rectangles and circles and calculating the area of each shape using measurements of length and width or diameter. For each shape, we multiplied surface area by average depth (calculated by averaging at least three depth measurements taken haphazardly within the shape). We summed the volumes of the component shapes to estimate total volume of each pool.

Statistical Analysis
To analyze factors influencing species richness, we performed a stepwise multiple regression analysis of species richness against all parameters (salinity, height above ocean, average pool volume, refuge index, distance from ocean) using the Akaike Information Criterion (AIC) to select the most parsimonious model (Cavanaugh 2007). We excluded from the analysis all pools that were completely dry on the second day of species counts because counts could not be made at or below waterline in waterless pools. We averaged data across the two sampling dates. Average species richness, average pool volume, refuge index, salinity, and height above ocean were
square-root transformed to meet the assumption of normality.

To test which factors affected species evenness, we performed a stepwise multiple regression analysis using all the factors (salinity, height above ocean, average pool volume, refuge index, distance from ocean) and selected the most parsimonious model using AIC values. For salinity, we also performed a post hoc power analysis within the stepwise regression model to determine the number of samples needed to detect significant results. We performed the power analysis on salinity because it was included in the final model based upon AIC score but was not statistically significant. Similar to the analysis for species richness, all dry pools were excluded. Average pool volume, refuge index, salinity, and height above ocean were square-root transformed to meet the assumption of normality.

To analyze habitat stability based upon pool volume, we performed a regression analysis of percent change in water volume against pool volume on the first day of measurements. Percent change in water volume and original pool volume were square-root transformed to meet the assumption of normality. We used JMP 10.0 for all analyses.

RESULTS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Slope</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool height</td>
<td>-2.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Average water volume</td>
<td>0.91</td>
<td>0.03*</td>
</tr>
<tr>
<td>Salinity</td>
<td>-0.67</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 1. Slope estimates of parameters included in the final model of average species richness as determined using AICc values. Stars indicate significant P-values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Slope</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pool height</td>
<td>-3.52</td>
<td>0.006*</td>
</tr>
<tr>
<td>Salinity</td>
<td>-2.27</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

Table 2. Slope estimates of parameters included in the final model of average species evenness as determined using AICc values. Stars indicate significant P-values.

Pool height above the ocean, average volume, and average salinity were included in the best stepwise regression model of average species richness (adjusted $r^2 = 0.75$, $F_{3,10} = 19.89$, $P < 0.0001$). The AIC score of the best model was 28.6 while the model including all parameters had an AIC score of 39.2. Species richness increased as pool height above sea level decreased and also increased as average water volume increased (Table 1, Fig. 1A, B). Richness tended to increase as salinity decreased (Table 1). Salinity was included in the final model although it did not explain a significant amount of variation in species richness, likely due to low statistical power ($P = 0.42$). A post-hoc power analysis revealed that increasing sample size to 25 would have yielded an 80% chance of detecting a significant effect of salinity.

Pool height and salinity were included in the final model of average species evenness (adjusted $r^2 = 0.45$, $F_{2,10} = 8.79$, $P = 0.002$). The AIC score of the best model was -9.1 while the model including all parameters had an AIC score of 1.8. Species evenness increased as pool height above sea level decreased and as salinity decreased (Table 2, Fig. 1C, D).

Pools with larger original volumes had a smaller percent decrease in volume after two days than did smaller pools (slope = -2.28 ± 0.88, $P = 0.02$, $r^2 = 0.27$).

DISCUSSION

We found that pool height above the ocean was the strongest factor driving species richness and evenness in tidal pools. Pool height may affect the connectedness of pools to the ocean—the source of many tidal pool organisms—as higher pools are likely inundated less frequently and receive less water volume from wave action and tides. Pool distance from ocean was another measure of connectedness but it did not affect evenness or richness. Horizontal distance from the ocean may represent a relatively small barrier to ocean waves in comparison to height above the ocean since water may move more easily over a long flat shore than a short steep one, especially in a region where the difference in ocean height between low tide and high tide is small. Thus, higher connectivity of our tidal pools (islands) to the ocean (mainland), represented by lower pool height, resulted in increased species richness and evenness as predicted by the theory of island biogeography. The connectedness of fragmented habitats to the mainland species source was the most important factor in maximizing these two components of species diversity.

Tidal pool size was also a driver of species richness; pools with larger water volumes supported more species than did small pools. Larger pools may provide a bigger target for
waves that bring colonizing species and may therefore experience higher immigration rates as predicted by the theory of island biogeography (MacArthur and Wilson 2001). Furthermore, larger pools may provide more stable and resilient habitats than small ones. For example, pools with larger initial volumes experienced smaller percent change in volume after two days, indicating that these pools were more resistant to desiccation. Stable habitats may exist for longer periods of time and therefore accumulate more species, increasing species richness. However, evenness did not increase with increasing volume. Relatively larger pools were able to support larger species such as juvenile fish and crustaceans, but the small size of the pools may have still limited their abundances. Therefore, accumulating more species did not necessarily reduce the dominance of more abundant species such as zebra nerites \((Puperita pupa)\) and dwarf brown periwinkles \((Littorina mespillum)\).

Higher pool salinity resulted in decreased species evenness. Pools with the highest salinity often contained only snail species such as beaded periwinkles \((Tectarius muricatus)\), zebra periwinkles \((Littorina ziczac)\), and zebra nerites, which can resist desiccation in the absence of water by sealing their shell with their operculum (Witherington and Witherington 2007). In pools with lower salinity, greater evenness suggested that organisms adapted to ocean or near-ocean salinity (e.g. fish, urchins, sea slugs, some species of snails) were present in addition to the organisms that can tolerate high levels of desiccation. It is

Figure 1. Species richness and evenness within tidal pools were influenced by pool height, salinity and volume. (A) Average species richness decreased with height above ocean. (B) Average species richness increased with average pool volume. (C) Average species evenness declined as pool height increased. (D) Average species evenness decreased as salinity increased.
possible that other species of microalgae grazers less tolerant of high salinity and desiccation competed with these desiccation-tolerant snails. Competition by species adapted to lower salinity may have prevented large numbers of these snails from dominating the habitat as they did in higher salinity areas, therefore increasing species evenness. Further study could investigate the presence of competition between snails and other species in tidal pools by removing low-salinity species and measuring changes in numbers of desiccation-tolerant snails present. Additionally, we found no evidence that species evenness was driven by the intermediate disturbance hypothesis as no optimal intermediate level of salinity maximized evenness; instead, species evenness declined linearly with salinity. It is possible that ocean or near-ocean salinity did not function as a disturbance to tidal pool organisms.

Our study has implications for the most important factors to consider when planning biological reserves. Our results suggest that connecting reserves to source populations increases species richness and evenness. For example, many marine protected areas (MPAs) have been created to release harvested marine organisms from harvesting and to enable the recovery of their populations (Sanchirico and Wilen 2001). While MPAs are often located around coral reefs, our study suggests the importance of creating links to seagrass beds, mangroves, the open ocean and other source populations. While many nature reserves do not have an obvious source of organisms, connections between fragments may also be important for dispersal between fragments. Metapopulation theory assumes that a habitat consists of a series of small patches with no mainland connection as in island biogeography, and suggests that species will become extinct once habitat fragmentation increases above a critical threshold. However, corridors between fragments allow for increased dispersal and may decrease the risk of extinction (Harrison and Bruna 1999). Additionally, while connectedness of habitats to source populations may increase species diversity, our results suggest that increasing the size of habitats may also be important for maximizing species richness. Larger reserves may accumulate more species over time and may be more stable habitats, resisting large disturbances that could threaten all species aside from the most disturbance-tolerant ones.

Our study highlights the interplay of factors contributing to species diversity. Understanding the most significant drivers of species diversity will aid in the implementation of effective conservation measures in fragmented habitats. Managing these drivers to maximize species diversity with limited space and resources is increasingly important as the global rate of species decline continues to escalate.

ACKNOWLEDGEMENTS
We thank the staff and crew of the Central Caribbean Marine Institute, especially Perry Oftedahl, for all of their support, and Z. Gezon, B. Taylor, and R. Chaves-Ulloa for their guidance and assistance in manuscript review.

AUTHOR CONTRIBUTIONS
All authors contributed equally. Order of authorship was decided using a random number generator.

LITERATURE CITED
Little Cayman


TURTLE GRASS GROWTH RESPONSE TO HERBIVORY

AMELIA F. ANTRIM, SETH A. BROWN, ELIZA W. HUNTINGTON, AND MOLLY R. PUGH

Faculty Editor: Brad Taylor

Abstract: Plants have evolved many ways to mitigate the effects of herbivory. Herbivory has been shown to increase growth rate in some terrestrial grasslands in a process known as compensatory growth. While studies have found differences in the level of herbivory that maximizes growth, compensatory growth is a well-established plant response in terrestrial systems. Though many studies have investigated compensatory growth in terrestrial plants, this phenomenon remains largely unexamined in marine systems. We tested compensatory growth as a response to herbivory in turtle grass (Thalassia testudinum), a common seagrass found along shorelines in the Caribbean. To simulate herbivory we clipped the tip of each blade. To stimulate mineralization and mimic rhizomatic carbon release as a mechanism of compensatory growth, we injected a sugar solution into the roots of unclipped shoots. We measured plant growth over the course of four days. We found that simulated herbivory had no effect on the growth rate of turtle grass, indicating that turtle grass does not exhibit short-term compensatory growth. However, we found that turtle grass has a rapid growth rate, which may be another mechanism by which this plant tolerates herbivory. Mechanisms such as rapid growth, which allow plants to make up for losses due to grazing, can shed light on herbivory tolerance in marine systems.

Key words: compensatory growth, herbivory tolerance, Thalassia testudinum, seagrass beds

INTRODUCTION
Primary producers have managed to thrive by evolving many mechanisms of defense against, and tolerance to, herbivores. Hairston, Smith, and Slobodkin (1960) attempted to explain the abundance of primary producers in the face of herbivory, sparking widespread debate between the importance of top-down and bottom-up herbivore regulation. In opposition to top-down regulation, theories on plant resistance to herbivory state that most plant material is not available to herbivores, as some plants contain inedible parts or produce defenses (Murdoch 1966). Alternatively, instead of preventing herbivory, some plants can tolerate herbivory by regrowing or reproducing in response to damage (Strauss and Agrawal 1999). Valentine (1997) and Strauss and Agrawal (1999) proposed five primary mechanisms by which plants mitigate losses to herbivory: (1) excess nutrient storage in roots, (2) consistently high growth rates, (3) higher photosynthetic rate after damage, (4) ability to reallocate energy and reserves from undamaged to damaged tissues post-grazing, and (5) increased biomass production to replace lost tissue after herbivory. In the last mechanism, known as compensatory growth, a plant responds to tissue damage by increasing its overall growth rate (McNaughton 1983).

Compensatory growth is a well-documented phenomenon in terrestrial systems. For example, growth rates of grassland vegetation in Yellowstone National Park were 47% higher in grazed versus ungrazed areas (Frank and McNaughton 1993). Likewise, grazing in Serengeti National Park was found to increase forage production rate (McNaughton 1985). One mechanism of compensatory growth is the release of carbon by damaged plants via root exudates, which stimulates soil microbes that can fix more nitrogen or mineralize soil nutrients (Hamilton and Frank 2001). Studies have found species-specific compensatory growth responses to varying levels of herbivory, indicating that plants require different herbivory rates to optimize growth (McNaughton 1983, Paige 1992).
Though many studies have investigated compensatory growth in terrestrial plants, this phenomenon remains largely unexamined in marine systems (Valentine et al. 1997), despite the importance of seagrasses for invertebrates, fish, and turtles (Bjorndal 1980). Studies of compensatory growth in sea grasses have yielded contradictory results, highlighting the need for further investigation (Valentine et al. 1997).

We tested compensatory growth in marine plants using turtle grass (*Thalassia testudinum*), an angiosperm found along coastlines in the Caribbean Sea. If turtle grass shows compensatory growth, plants experiencing simulated grazing should have higher growth rates than ungrazed plants. Furthermore, if the stimulation of microbe nitrogen fixation by carbon-rich root exudates is the mechanism for compensatory growth in turtle grass, then carbon injections into the root mass of ungrazed turtle grass should increase growth rate. Alternatively, if turtle grass does not use compensatory growth as a response to herbivory, growth rate will not increase in response to simulated herbivory or organic carbon additions, and thus turtle grass may use other mechanisms for tolerance to herbivory.

**METHODS**

To test marine plant growth rate in response to herbivory, we simulated herbivory on turtle grass plants in a seagrass bed in South Hole Sound, Little Cayman Island, in March 2013. We selected 100 shoots in two parallel transects, with each shoot one meter from neighboring shoots. We selected all plants at a uniform depth of 0.5 m at high tide to control for variability in temperature, light, and dissolved oxygen, and the water was deep enough to prevent desiccation at low tide. Density of turtle grass cover was relatively consistent. On each focal turtle grass shoot, we poked a hole at the base of all blades as a marker for growth based on the methods of Short and Duarte (2001), and tagged the shoot with flagging tape. To simulate herbivory, we clipped one cm off the tip of each blade of selected shoots. Additionally, to understand whether compensatory growth occurs via carbon release from roots, we injected a 25 mL sugar solution into the soil at the base of a group of unclipped shoots. In an attempt to ensure response to sugar, we used a high concentration solution of 0.12 molar sucrose. Individual shoots were randomly assigned to one of five treatments: control (no clipping), clipped once, clipped every other day, clipped every day, and sugar injection (20 plants per treatment). Four days after the initial cuts and sugar injections, we measured the height of the punched hole above the blade base to determine growth. We counted number of blades and measured initial height and width of the longest blade as potential covariates.

**RESULTS**

Blade growth rate did not differ among treatments ($F_{4,84} = 0.54, P = 0.70$, Fig. 1, Table 1). Because covariates (number of blades, initial shoot height and shoot width) did not affect the growth rates, we excluded them from the final model.

![Figure 1. Growth rate across all treatments was not significantly different. Clipping treatments simulated herbivory by removing ~ 1 cm from each blade. Sugar was added as a labile source of organic carbon.](image-url)
Table 1. Mean, standard error, and range of the growth rate, height, width, and number of blades across all treatments.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard Error</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Rate (mm/day)</td>
<td>2.34</td>
<td>0.09</td>
<td>0.58</td>
<td>4.85</td>
</tr>
<tr>
<td>Height (mm)</td>
<td>194.1</td>
<td>5.5</td>
<td>100</td>
<td>320</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>8.71</td>
<td>0.16</td>
<td>4.8</td>
<td>12.2</td>
</tr>
<tr>
<td>No. of Blades</td>
<td>4</td>
<td>0.09</td>
<td>3</td>
<td>9</td>
</tr>
</tbody>
</table>

Discussion

Turtle grass growth rate was similar across all treatments, indicating that during short time periods turtle grass does not exhibit compensatory growth in response to these levels of herbivory. Because the growth rate of turtle grass did not increase with added carbon (i.e., the sugar injection), turtle grass stimulation of soil microbes may not be an important compensatory mechanism. Even if the roots do not have the capacity to secrete carbon, sugar injections should stimulate nitrogen-fixing bacteria in a carbon-poor environment; the lack of response to the added carbon suggests that benthic microbes may already be carbon-saturated and provide sufficient amounts of nitrogen to turtle grass. Dead seagrass blades are carbon-rich, providing high levels of nutrients to microbes via detrital pathways (Koike et al. 1987); additional carbon inputs to a high-carbon system may not be necessary. Therefore, additional carbon excretion from root exudates following herbivory may not benefit the plant, as our results show that additional carbon influx did not increase growth rates.

Although the turtle grass growth rates were not reduced by herbivory in this study, future work should examine how sustained removal of photosynthetic tissue affects long-term plant growth. Few studies have investigated natural herbivory on turtle grass. For our low herbivory treatment we chose to clip one cm once during our four-day sampling period, which was approximately equal to mean blade growth. Given that our highest simulated herbivory rate was four times the mean growth rate, sustaining this level of herbivory may deplete the plant’s aboveground biomass. Turtle grass rhizomes contain carbohydrate and protein stores that can be translocated to photosynthetic tissue when aboveground biomass has been removed (Valentine et al. 1997). Future studies could determine natural herbivory rates on turtle grass, and examine whether sustained herbivory negatively affects turtle grass growth rate, productivity, or reproduction by eventually depleting rhizomatic nutrient and energy stores.

Though we did not find evidence for compensatory growth in turtle grass, this plant may still exhibit other mechanisms for tolerance to herbivory. Turtle grass evolved under high grazing pressure from large vertebrates, such as dugongs, manatees, and turtles, which have since declined in abundance (Heck and Valentine 2006). As such, turtle grass likely developed a high growth rate (Thomas et al. 1961), a property which confers resistance to herbivory. We found that new blades grow as much as five mm per day. In addition to increasing tolerance to herbivory (Strauss and Agrawal 1999), high growth rates contribute to fast blade turnover rate in turtle grass (Tomasko et al. 1996). High blade turnover provides consistent nutrient cycling in the substrate to allow for rapid turtle grass growth.

Given that compensatory growth is not present in turtle grass over short periods of simulated herbivory, our study suggests that another mechanism such as rapid growth may account for herbivore tolerance in this species. Turtle grass, abundant throughout tropical marine ecosystems, comprises an integral part of coastal reef systems and is important for the maintenance of biodiversity in these areas. Understanding the mechanisms by which turtle grass persists despite pressure from herbivory may shed light upon the dynamics of this ecosystem, informing our understanding of herbivore tolerance in marine ecosystems.
ACKNOWLEDGMENTS
We would like to thank the staff at the Little Cayman Research Center for allowing us to use their facilities. We would also like to thank R. Chaves-Ulloa, Z. Gezon, and B. Taylor for their assistance in methods and revision.

AUTHOR CONTRIBUTION
All authors contributed equally.

LITERATURE CITED