PARTICIPANTS OF THE DARTMOUTH BIOLOGY FSP 2017

FACULTY
MATTHEW P. AYRES  MARK E. LAIDRE
HANNAH TER HOFSTEDE  CELIA Y. CHEN

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LOUISE J. BARIAS  LEIGH M. MOFFETT
ERIC D. BRUNNER  HANNAH M. NASH
REBECCA W. FLOWERS  EMILY B. OKUN
SAMUEL R. GOCHMAN  LEAH VALDES
FRANCESCA C. GOVERNALI  MARIKO C. WHITENACK

VOL. 27 PUBLICATION EDITOR
CALLUM H. BACKSTROM
~ From Santa Rosa ~
Back, left to right: Madi Gamble, Leah Valdes, Hannah Nash, Louise Barias, Leigh Moffett, Rebecca Flowers, Callum Backstrom, Sam Gochman, Braden Elliott, Eladio, Mark Laidre
Front, left to right: Emily Okun, Gabby Lowry, Mari Whitenack, Eric Brunner, Caroline Koller, Francesca Governali

~ From El Campanario ~
Standing, left (left to right): Eric Brunner, Amber Ahronian, Hannah ter Hofstede, Hannah Nash, Rebecca Flowers, Leigh Moffett
Standing, back (left to right): Sam Gochman, Braden Elliott, Callum Backstrom, Leah Valdes, Matt Ayres, Madi Gamble, Emily Okun, Francesca Governali, Sharon Martinson, Laurel Symes
Sitting (left to right): Gabby Lowry, Caroline Koller, Mari Whitenack, Louise Barias

~ From Little Cayman ~
Back, left to right: Emily Okun, Francesca Governali, Sam Gochman, Callum Backstrom, Hannah Nash, Madi Gamble, Gabby Lowry, Amber Ahronian, Braden Elliott, Rebecca Flowers, Leigh Moffett, Leah Valdes, Celia Chen, Louise Barias
Front, left to right: Eric Brunner, Mari Whitenack, Caroline Koller
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 available online and hard copies are held in the Life Sciences Center at Dartmouth College and in the field stations that we visit.

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 order of authorship on papers is usually alphabetical or haphazard, because all authors contribute equally on projects. For each paper there is a faculty editor (indicated after the author listing), who takes responsibility for defining the revisions, and decides on the acceptability of manuscripts for publication. Graduate student Teaching Assistants are also heavily involved as mentors at every stage, from project design to final manuscript.

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 40 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 are grateful for the generous financial support of the Biology Foreign Studies Program from Dorothy Hobbs Kroenlein.

If you have questions about this volume or the program, contact the Biological Sciences Department at Dartmouth College, Hanover New Hampshire, USA. Currently, the Biology Foreign Studies Program Director is Matthew Ayres at Matthew.P.Ayres@dartmouth.edu and the administrative assistant is Sherry L. Finnemore, Sherry.L.Finnemore@dartmouth.edu.

Matt Ayres
Hanover NH, USA
28 Dec 2017
**Costa Rica 2017 Schedule**


<table>
<thead>
<tr>
<th>Date</th>
<th>Day</th>
<th>Location</th>
<th>Morning</th>
<th>Afternoon</th>
<th>Evening</th>
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<tbody>
<tr>
<td>4-Jan</td>
<td>Wed</td>
<td>To San Jose</td>
<td>Travel</td>
<td>Travel</td>
<td>Arrive in evening</td>
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<tr>
<td>5-Jan</td>
<td></td>
<td>In San Jose</td>
<td>OTS, Program overview</td>
<td>San Jose exploration</td>
<td>Group dinner in SJ</td>
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<tr>
<td>6-Jan</td>
<td></td>
<td>To Palo Verde</td>
<td>Travel to Palo Verde</td>
<td>Orientation. Research tactics 1</td>
<td>Lec: Intro CR ecol (MA,CB). Nightwalk</td>
</tr>
<tr>
<td>7-Jan</td>
<td></td>
<td>At PV</td>
<td>Orientation. Vertebrates (MG)</td>
<td>Tactics: questions. Arthropods (MA)</td>
<td>Tactic: Hypotheses &amp; predictions</td>
</tr>
<tr>
<td>8-Jan</td>
<td>Sun</td>
<td>At PV</td>
<td>SIP-1 research development</td>
<td>Plant lab (BE). SIP-1 proposals</td>
<td>Lec: Primates (ML, LV)</td>
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<tr>
<td>9-Jan</td>
<td></td>
<td>At PV</td>
<td>SIP-1 pilot studies</td>
<td>Tactics: 3: Statistics (MA). SIP-1 research</td>
<td>Tactic: Theories &amp; context.</td>
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<tr>
<td>10-Jan</td>
<td></td>
<td>At PV</td>
<td>SIP-1 research</td>
<td>SIP-1 research</td>
<td>Lec: Plant-herb (BE, GL). Writing lab.</td>
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<tr>
<td>11-Jan</td>
<td></td>
<td>At PV</td>
<td>SIP-1 research</td>
<td>SIP-1 research</td>
<td>SIP-1 symposium. SIP-1 v01 due</td>
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<tr>
<td>12-Jan</td>
<td></td>
<td>At PV</td>
<td>River trip</td>
<td>SIP-1 revisions</td>
<td>SIP-1 revisions; nightwalk</td>
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<tr>
<td>13-Jan</td>
<td></td>
<td>To Santa Rosa</td>
<td>Bus to trailhead. Hike to beach.</td>
<td>Orientation. Sea Turtles (MG)</td>
<td>Field: Sea turtle nesting</td>
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<tr>
<td>14-Jan</td>
<td></td>
<td>At SR</td>
<td>Orientation. Mangroves (BE)</td>
<td>Reconnaissance. Hermit crabs (ML)</td>
<td>Field: Sea turtle nesting</td>
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<tr>
<td>15-Jan</td>
<td>Sun</td>
<td>To Monteverde</td>
<td>Travel</td>
<td>Travel. Orientation.</td>
<td>Revisions of SIP-1; SIP-1 v02 due.</td>
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<tr>
<td>16-Jan</td>
<td></td>
<td>At MV</td>
<td>Orientation &amp; reconnaissance.</td>
<td>SIP-2 research development</td>
<td>SIP-2 proposals. SIP-1 accepted &amp; filed.</td>
</tr>
<tr>
<td>17-Jan</td>
<td></td>
<td>At MV</td>
<td>SIP-2 pilot studies</td>
<td>Hummingbird garden. SIP-2 research</td>
<td>Lec: Diversity &amp; coexistence (MG, EB)</td>
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<tr>
<td>18-Jan</td>
<td></td>
<td>At MV</td>
<td>SIP-2 research</td>
<td>SIP-2 research</td>
<td>SIP-2 research</td>
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<tr>
<td>19-Jan</td>
<td></td>
<td>At MV</td>
<td>SIP-2 research</td>
<td>Hummingbirds. SIP-2 research</td>
<td>Lec: Ecosystems (MA, FG)</td>
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<tr>
<td>20-Jan</td>
<td></td>
<td>At MV</td>
<td>Analysis &amp; writing</td>
<td>Analysis &amp; writing</td>
<td>SIP-2 symposium. SIP-2 v01 due.</td>
</tr>
<tr>
<td>22-Jan</td>
<td>Sun</td>
<td>To Cuerici</td>
<td>Travel to San Jose</td>
<td>Travel. Orientation.</td>
<td>Revisions of SIP-1; SIP-1 v02 due.</td>
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<tr>
<td>23-Jan</td>
<td></td>
<td>At Cuerici</td>
<td>Orientation</td>
<td>SIP-3 planning / proposals</td>
<td>Lec: Coevol 2 (HH, LM)</td>
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<tr>
<td>24-Jan</td>
<td></td>
<td>At Cuerici</td>
<td>SIP-3 research</td>
<td>SIP-3 research</td>
<td>SIP-3 research</td>
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<tr>
<td>25-Jan</td>
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<td>At Cuerici</td>
<td>SIP-3 research</td>
<td>SIP-3 research</td>
<td>Lec: Bats (HH, MW)</td>
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<tr>
<td>26-Jan</td>
<td></td>
<td>At Cuerici</td>
<td>SIP-3 research</td>
<td>SIP-3 analyses &amp; context</td>
<td>SIP-3 symposium</td>
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<tr>
<td>27-Jan</td>
<td></td>
<td>At Cuerici</td>
<td>SIP-3 v01 due</td>
<td>Reconnaissance. Writing. Revisions.</td>
<td>Writing. SIPS 1-2 accepted &amp; filed.</td>
</tr>
<tr>
<td>28-Jan</td>
<td></td>
<td>To El Campanario</td>
<td>Hike. Bus to Sierpe.</td>
<td>Boar to El Campanario</td>
<td>Diversity, katydid style (Laurel &amp; Sharon)</td>
</tr>
<tr>
<td>29-Jan</td>
<td>Sun</td>
<td>At El Campanario</td>
<td>Orientation</td>
<td>SIP-4 research development</td>
<td>SIP-4 proposal ideas</td>
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<td>30-Jan</td>
<td></td>
<td>At El Campanario</td>
<td>Orientation</td>
<td>SIP-4 plan</td>
<td>Lec: Social insects (MA, EO)</td>
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<tr>
<td>31-Jan</td>
<td></td>
<td>At El Campanario</td>
<td>SIP-4 pilot studies</td>
<td>SIP-4 research</td>
<td>Nightwalk</td>
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<td>1-Feb</td>
<td></td>
<td>At El Campanario</td>
<td>SIP-4 research</td>
<td>SIP-4 research</td>
<td>Lec: Cons Bio 1 (MA, SG)</td>
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<td>2-Feb</td>
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<td>At El Campanario</td>
<td>SIP-4 research</td>
<td>SIP-4 research</td>
<td>Lec: Cons Bio 1 (MA, SG)</td>
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<td>3-Feb</td>
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<td>At El Campanario</td>
<td>SIP-4 research</td>
<td>SIP-4 analyses &amp; context</td>
<td>SIP-4 symposium.</td>
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<tr>
<td>4-Feb</td>
<td></td>
<td>To Las Cruces</td>
<td>Boat out of El Campanario</td>
<td>Travel to Las Cruces</td>
<td>Revisions. Writing.</td>
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<tr>
<td>5-Feb</td>
<td>Sun</td>
<td>At Las Cruces</td>
<td>Orientation.</td>
<td>Revisions. Writing. Botany.</td>
<td>Lec: Avian Ecol (MG, HN)</td>
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<td>7-Feb</td>
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<td>At Las Cruces</td>
<td>Revisions. Writing. Botany.</td>
<td>Writing. Botany. practicum.</td>
<td>SIPs 1-4 all accepted and filed.</td>
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<td>8-Feb</td>
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<td>To La Selva</td>
<td>Travel</td>
<td>Travel</td>
<td>Lec: Behav 1 (HH, RF)</td>
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<tr>
<td>9-Feb</td>
<td></td>
<td>At La Selva</td>
<td>Orientation</td>
<td>SIP-5 research development</td>
<td>SIP-5 proposals. Night walk</td>
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<tr>
<td>10-Feb</td>
<td></td>
<td>At La Selva</td>
<td>SIP-5 pilot studies</td>
<td>SIP-5 research</td>
<td>Lec: Behav 2 (HH, CK)</td>
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<tr>
<td>11-Feb</td>
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<td>At La Selva</td>
<td>SIP-5 research</td>
<td>SIP-5 research</td>
<td>SIP-5 analyses</td>
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<tr>
<td>12-Feb</td>
<td>Sun</td>
<td>At La Selva</td>
<td>SIP-5 research</td>
<td>Agroecology field trip</td>
<td>Lec: Cons Bio 2 (BE, AA)</td>
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<td>13-Feb</td>
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<td>At La Selva</td>
<td>SIP-5 research</td>
<td>SIP-5 analyses &amp; context</td>
<td>SIP-5 symposium.</td>
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<td>14-Feb</td>
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<td>At La Selva</td>
<td>Writing SIP-5 v01 due.</td>
<td>SIP-5 v02, v03. filed.</td>
<td>Final deadline for Costa Rica papers</td>
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<tr>
<td>15-Feb</td>
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<td>To San Jose</td>
<td>Travel to San Jose.</td>
<td>Swap gear at OTC. Recon in San Jose</td>
<td>Prepare for Caribbean</td>
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<tr>
<td>16-Feb</td>
<td></td>
<td>To Grand Cayman</td>
<td>Depart Hotel 04:30. Fly to Miami</td>
<td>Fly to Grand Cayman</td>
<td>Overnight in Grand Cayman</td>
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<tr>
<td>17-Feb</td>
<td></td>
<td>To Little Cayman</td>
<td>Fly to Little Cayman</td>
<td>Arrive Little Cayman Research Center</td>
<td>Orientation</td>
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<tr>
<td>18-Feb</td>
<td></td>
<td>To Miami</td>
<td>Fly to Grand Cayman</td>
<td>Fly to Miami. End of program.</td>
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<td>Date</td>
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<td>17 Feb</td>
<td>Group Arrives at 3:20pm and 5:35pm from Grand Cayman</td>
<td>Unpack and have dinner</td>
<td>Introduction to LCRC (PQ)</td>
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<td>Fri</td>
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<td>Main orientation</td>
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<td><strong>Discussion:</strong> program to date and expectations for LC segment.</td>
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<td>18 Feb</td>
<td>Orientation and General natural history</td>
<td>Lecture: Coral reefs in the Caymans (CC) Safety instruction and gear assigning Snorkeling in back reef</td>
<td>SCUBA and Snorkeling Shore dive at Cumber's Cave</td>
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<td>Sat</td>
<td></td>
<td><strong>Queen Conch Project</strong></td>
<td><strong>Queen Conch Project</strong></td>
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<td>Introduction to Queen Conch (CC)</td>
<td>Introduction to Queen Conch (CC)</td>
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<td>Lecture: Fish behavior (MG) 7pm</td>
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<td>8pm Jim Hellemm</td>
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<td>Creature feature</td>
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<td><strong>Film:</strong> Secret sex life of fish</td>
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<td>19 Feb</td>
<td>Queen Conch project</td>
<td>Queen Conch Project</td>
<td>Queen Conch Project</td>
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<td>Sun</td>
<td></td>
<td>Data collection design</td>
<td>Snorkeling at South Hole</td>
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<td><strong>Queen Conch Project</strong></td>
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<td>Data collection design</td>
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<td>20 Feb</td>
<td>Queen Conch project</td>
<td>Queen Conch Project</td>
<td>Critique: Invertebrates (Carolyn Koller)</td>
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<tr>
<td>Mon</td>
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<td>Lecture: Invertebrates and zooplankton (CC)</td>
<td>Data analysis and findings</td>
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<td>21 Feb</td>
<td>Project 1 begins</td>
<td>SCUBA and Snorkeling 9am Queen Conch Project Presentation</td>
<td>Project 1 Brainstorm and exploration</td>
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<td>Tues</td>
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<td>Project 1 data collection</td>
<td>Lecture: Grouper Moon (Bryce Semmens)</td>
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<td>22 Feb</td>
<td>Project 1</td>
<td>Project 1 data collection</td>
<td><strong>Project 1 Proposal</strong></td>
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<tr>
<td>Wed</td>
<td><strong>Critique:</strong> Zooplankton (Amber Ahronian) Finalize project 1 idea, design, and group members</td>
<td>Project 1 idea discussion and proposal development</td>
<td>Project 1 Proposal</td>
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<td><strong>Project 1 Proposal Presentation</strong></td>
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<td>Lecture: Reef Nekton (CC)</td>
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<td><strong>Critique:</strong> Coral-algae competition (Mariko Whitenack)</td>
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<td>23 Feb</td>
<td>Project 1</td>
<td>Project 1 data collection</td>
<td>Critique: Fish ecology (Hannah Nash) 5PM</td>
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<td>Thurs</td>
<td><strong>Project 1 Proposal due</strong></td>
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<td><strong>Discussion:</strong> Graduate School/Career</td>
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<td><strong>Critique:</strong> Herbivory (Emily Okun) Project 1 data collection</td>
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<td>Algae module</td>
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<td><strong>Critique:</strong> Fish Biology (Louise Barias)</td>
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<td>24 Feb</td>
<td>Project 1</td>
<td>Project 1 5 pm Carrie Manfrino lecture</td>
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<td>Fri</td>
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<td>25 Feb Sat</td>
<td>Project 1</td>
<td>SCUBA and Snorkeling</td>
<td>RR: Party at the Iguana</td>
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<td>Critique: Coral Reef Bleaching (Leigh Moffett)</td>
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<td>Project 1 – writing and analysis</td>
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<tr>
<td>26 Feb Sun</td>
<td>Dia libre (OFF)</td>
<td>Beach Cleanup</td>
<td>RR: Oscar night</td>
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<td>Cookout and snorkeling at Point of Sand</td>
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<tr>
<td>27 Feb Mon</td>
<td>Project 1 completion</td>
<td><strong>Project 1 PRESENTATIONS</strong></td>
<td>Celia’s Talk at Southern Cross</td>
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<tr>
<td></td>
<td>Project 2 begins</td>
<td>Project 1 – 1st Draft due</td>
<td>Project 1 refine drafts</td>
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Dartmouth Studies in Tropical Ecology  
Curso de Biología Tropical, Universidad de Dartmouth

Research in the Tropics I and II: 4 January – 14 February 2017  

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Callum H. Backstrom, Samuel R. Gochman, Caroline V. Koller,  
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SIZE ISN’T EVERYTHING: THE ROLE OF BODY SIZE AND TRAP STEEPNESS IN ANTLION HUNTING SUCCESS

GABRIELA LOWRY, ERIC BRUNNER, LEAH VALDES, LOUISE BARIAS, AND AMBER AHRONIAN

Faculty Editor: Matthew Ayres

Abstract: Other things being equal, predators are selected to maximize hunting efficiency such that the energetic benefits of prey capture maximally outweigh the energetic costs of hunting. Some predators hunt by building traps. In this strategy, the predator typically waits for prey to enter the trap before initiating contact. Understanding both how the trap is built and how the predator captures prey that enters can provide insight into the costs, benefits, and mechanisms of alternative strategies. We investigated hunting strategies of the antlion (Myrmeleon crudelis), an insect that builds sand pits as larvae to trap ants. We investigated relationships between hunting success and the body size of antlions and ants. We also measured the effect of the trap’s physical dimensions on hunting success. For the experiment, we fed antlions either large (> 5 mm) or small (< 3 mm) ants and recorded whether or not they successfully killed the ant. Due to their hunting strategy, we expected larger antlions to be more successful at killing large ants. Although our data supported this relationship, we were surprised to find that hole steepness was a markedly better predictor of hunting success with large ants. This result suggests that, at least for antlions, the energy investments in trap architecture apparently influence their hunting efficiency.

Key words: ambush, predation, trap architecture

INTRODUCTION

Predation is an important source of energy transfer in ecosystems. When successful, the predator benefits from a gain in energy that outweighs the energetic costs of acquiring that prey; when unsuccessful, the energetic costs outweigh the benefits, and predators have low reproductive success. Thus, there is selection for predators to evolve strategies that maximize hunting efficiency. Some different hunting strategies can be found in nature. These include pursuit predation and sit-and-wait predation. The former pursue their prey until it escapes or is captured, while the latter remain in one location and wait for prey to approach within striking range. One strategy of sit-and-wait predators is trap use, in which the predator captures and kills its prey in either a constructed or natural trap. This is found, for example, in web-building spiders, pitcher plants, and anglerfish. Studying trap-building tactics may provide insight into the evolution of predation strategies that maximize hunting efficiency.

Antlions (Neuroptera: Myrmeleontidae) are fiercely aggressive trap-building predators in their larval state. After winged adults deposit eggs, the antlion larvae hatch and dig funnel-shaped holes in sandy substrate. The antlion digs the hole by catapulting sand and other debris from the pit with its head. When a small invertebrate enters the pit, its weight causes the sand beneath it to crumble so that it falls down the incline toward the bottom, where the antlion senses the vibrations and grasps the prey in its mandibles. The antlion then attempts to maintain its hold on the prey while injecting it with digestive fluids until the invertebrate either dies or escapes. If the antlion has successfully killed the invertebrate, it will drag the prey under the sediment where it will be consumed then or later.

Antlions are well suited for studies of hunting efficiency in trap-building predators because they have a global distribution and inhabit a wide variety of ecosystems. In addition, their prey (ants and other small invertebrates) are highly abundant and easily manipulated, their hunting process is quick, and they employ a unique trap-building hunting strategy.

In this study, we investigated the effects of antlion size, prey size, hole shape, and hole size on antlion hunting success. The antlion’s
hunting strategy requires that it forcibly hold the ant in the trap long enough to inject sufficient digestive enzymes; therefore it is plausible that antlion hunting success is influenced by the body sizes of both predator and prey. If this is true, larger antlions will have greater hunting success than smaller antlions. Alternatively, it is possible that the slope of the hole determines hunting success by making it more difficult for the ant to escape. In this case, antlions in holes with steeper slopes will have greater hunting success. Alternatively, body size and hole steepness might be largely unrelated to antlion predation success. Factors other than antlion body size and hole steepness may have a larger influence on the probability of hunting success.

METHODS

Field Experimental Procedure
We captured ants of two different size classes using an aspirator outside the Organization for Tropical Studies research station in Palo Verde. The small ants were under 3 mm in length and the large were over 5 mm. During 9-11 January 2017, we observed the behavioral response of wild antlions to small and large ants. First, we measured the depth and width of the antlion traps using calipers. With forceps, we released a single small or large ant into the trap. We recorded if the antlion attacked or successfully dragged the ant under the sediment. Once the ant had either escaped or been dragged under the sand surface, we extracted the antlion using a tablespoon and measured its length from pincers to posterior using calipers. We tested each animal only once.

We used lab experiments to supplement our field data, which tended to under-represent small antlions. We first collected sediment and antlions from the side of the road. To do so, we scooped out the sediment from the bottom of the antlion traps and sifted through it using a 250 or 500 μm sieve to find the antlion. We then homogenized the sediment before distributing it 3-cm deep into three trays, one corresponding to each of three antlion size classes (< 6.5mm, 6.5-8mm, > 8mm). Twelve antlions from each size class were placed into their respective trays and allowed to dig burrows overnight. Extra antlions were placed in a fourth tray. We then repeated our field experimental procedure on the captive antlions, randomly matching each trap with either a small or large ant. In doing so, we were able to ensure that we had an approximately uniform distribution of antlion size in our overall dataset. We timed each of the lab encounters after noticing that larger ants took longer to kill in the field.

Statistical Analyses
Using JMP Pro V. 13.0, we created a generalized linear model with a binomial link function to determine if there was a relationship between antlion size (as a continuous variable) and probability of successful capture. The model also included ant size as a categorical variable (large or small) and antlion size crossed with ant size. We used the same model to evaluate the relationship between steepness of antlion traps (calculated from width and depth measurements) and probability of success, substituting steepness for antlion size. Using the Akaike Information Criterion (AIC), we compared the ability of these two models (with either antlion size or hole steepness) to predict capture success. We used a linear regression to characterize the relationship between hole steepness and antlion size.

RESULTS
For the 44 antlions presented with large ants, there was a positive relationship between antlion body size and probability of capture success (chi-square = 4.13, P = 0.04); that is, larger antlions had a higher probability of killing the large ants than did smaller antlions (Figure 1). Among the antlions presented with small ants, there was no higher capture success and no relationship between antlion body size and probability of success; 83% of antlions sampled were able to capture the small ants across all body sizes (chi-square for effect of antlion body size = 0.25, P = 0.61).
For antlions presented with large ants, there was a positive relationship between hole steepness and capture success, indicating that antlions with steeper traps were significantly more successful at capturing larger ants than those with shallower traps (chi-square = 6.69, \( P = 0.009 \); Figure 2). When antlions were presented with small ants, steepness had no significant effect on capture success (chi-square = 0.07, \( P = 0.79 \)).

Figure 1. When the prey were large ants (top), larger antlions had a higher capture success rate than small antlions, but body size had no significant effect on antlions presented with small ants (bottom).

When the prey were large ants, hole steepness was a better predictor of capture success than antlion body size (delta AIC = -6.48). Antlions with steeper traps were more successful at killing large ants than were large antlions in general; that is, hole geometry was a better predictor of success than body size alone.

Antlion size and hole steepness were positively correlated. Larger antlions had steeper holes: at about 4 (± 0.4)°/mm of body size (\( P < 0.001, r^2 = 0.62 \)). Additionally, the variances for hole steepness increased as antlion size
increased, indicating that larger antlions were able to, but didn’t always, produce steeper traps (Figure 3).

DISCUSSION

Our results revealed clear relationships among antlion body size, hole steepness, and the probability of successfully capturing large ants. Larger antlions tended to dig steeper traps, which had higher capture success.

Presumably, larger antlions are more capable of maintaining steeper, and therefore more effective, holes than smaller antlions. A possible explanation is that larger antlions have the physical strength to more frequently flip sand from their burrows, thereby maintaining traps that were steeper than the angle of repose. The apparent pattern of increasing variance in hole steepness for large antlions (Fig. 3) suggested that large antlions are able to build steeper holes, but do not always do so.

The relatively large variance in the steepness of holes built by large antlions is particularly curious because, surprisingly, hole steepness was a more important determinant of predation success than body size. This complicates the relationship between body size and hunting success. It is possible that the relationship between antlion body size and hunting success is largely an artifact of the relationship between antlion body size and hole steepness. Perhaps there is an adaptive reason that large antlions do not all build steeper traps to increase predation success. Future research could investigate the circumstances under which large antlions produce shallower traps, despite their apparent ability to create steeper traps. Perhaps antlions do not invest as much energy in creating steeper traps when the regional density of prey is high, visitation is more frequent, and the probability of capturing any particular ant is less important.

Our study sheds some light on the significance of trap architecture for sit-and-wait predators in general, who invest medium to large amounts of energy in building structures that increase their predatory success. Plasticity in predator trap structures may permit predators to maximize the energetic efficiency of their trapping in varying circumstances.

ACKNOWLEDGEMENTS

We would first like to thank Professor Ayres for introducing us to antlions in Palo Verde and providing support and advice through the entirety of the project. We would also like to thank Madi Gamble and Braden Elliott for their constant support and for providing feedback on multiple variations of this manuscript. We want to thank Romelio for providing sustenance over our time at the station, and the antlions for being fascinating and cooperative study subjects. Finally, we would like to thank the entire FSP group of 2017 for being an incredibly enjoyable group of peers to work with.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this project.
GUMBOS IN LIMBO: PHOTOSYNTHESIS IN THE BARK OF *BURSERA SIMARUBA*

EMILY B. OKUN AND HANNAH M. NASH

Faculty Editor: Matt Ayres

Abstract: Key evolutionary innovations may explain why some organism types are more successful at surviving and diversifying across wide geographic ranges. Photosynthetic bark could function as such a key innovation in *Bursera* species by allowing for some photosynthesis during the dry season when leaf senescence fully halts photosynthesis in competing trees. We tested for photosynthetic bark in *Bursera simaruba* (gumbo limbo tree) by comparing the sugar content of the bark of nine trees under differing levels of light exposure: sunny, shady, and experimentally light-deprived. Bark samples from each treatment area were lysed and suspended in water to dissolve cellular sugars into solution, which was then analyzed using a Brix Refractometer. The experimentally light-deprived treatment contained about 35% less sugar than the unmanipulated sunny or shady sides of trees. Our results suggest that photosynthesis occurs in the bark of *B. simaruba*, which is consistent with the hypothesis that photosynthetic bark has been a key evolutionary innovation that has contributed to the adaptive radiation of *Bursera* in the seasonally dry regions of the Neotropics.

Key words: adaptive radiation, bark, *Bursera*, key innovation, photosynthesis

INTRODUCTION

Some organism types are more successful at surviving and diversifying across wide geographic ranges than others. Successful adaptive radiations within a clade are theorized to be the result of key innovations. For example, cichlid fish possess a second pair of jaws that are highly variable among species, and are cited as the reason for cichlids’ success across a wide range of habitats and the high level of species richness within the group (Meyer 2015). A key innovation opens up room for new species to fill a range of previously unexplored niches, leading to adaptive radiation.

The genus *Bursera* is a diverse and successful clade of over 100 species that occur in seasonally dry tropical forests ranging from Southern Florida through Northern Argentina, in some areas being the most common woody plant genus (Becerra and Venable 2008). All species share a few key traits: seasonal deciduousness, occurrence in dry climates, and layer of fleshy green bark which is thought to be photosynthetic. The green bark might provide a photosynthetic advantage and could be a key innovation contributing to the success of the *Bursera* clade over a broad geographic area. Photosynthetic bark would provide security during the dry season when leaf senescence halts photosynthesis in competing trees. A majority of plant water loss occurs through transpiration in leaves, making them costly to maintain during the driest part of the year. Its bark could allow *Bursera* to conserve water by getting rid of costly leaves and yet continuing to produce sugars.

Because photosynthesis is dependent on sunlight, lower light levels can decrease the rate of photosynthesis and lead to lower levels of sugar within cells. We tested for evidence of photosynthesis in the bark of *Bursera simaruba* (gumbo limbo tree or indio desnudo) by examining how the sugar content of bark cells responds to differing levels of light. We compared sugar levels between the sunny sides and shady sides of tree trunks, as well as areas of the trunks that were experimentally deprived of all light. If the bark photosynthesizes, then cells from sun-exposed areas of the trunk would be expected to contain more sugar than cells from shady areas, and from light-deprived areas. If the bark does not photosynthesize, then the sugar content of bark cells would be the same regardless of differing levels of light exposure across sample sites on each tree. We also used light microscopy to look for chloroplasts within bark tissue.

METHODS

Sample Collection

In Palo Verde National Park, Costa Rica, from January 9th to January 11th, nine *B. simaruba* trees were selected for sampling based on the presence of sun striking the trunk at chest height. If an area of the trunk was not clearly naturally
shaded, we used the southern side as the sunny side because Palo Verde National Park is in the northern hemisphere. To create completely light-deprived areas of tree bark, we taped a piece of cardboard to each tree in a sunny area at chest height. Approximately 24 hours later we took bark samples from each of the three treatment areas (sunny side, shady side, and experimentally light-deprived) around the trunk of each tree. To collect samples, we first peeled away the outer, flakey layer of red bark covering the green bark. We then used a knife to shave off the outer layer of green fleshy bark while minimizing contact with the red woody layer of the tree directly underneath the green bark. Roughly 0.3 g of bark was collected for each sample.

**Sample Preparation and Brix Refractometry**
Before preparing the bark samples, we created a standard curve using table sugar to test the calibration of the Brix Refractometer. This allowed us to approximate the percent sugar in each sample (Figure 1). To prevent observer bias, we then randomized the bark samples by covering the labels with opaque tape and shuffling them. We crushed each sample using a mortar and pestle until the bark was reduced to a fine green powder. Then we placed 0.14 g of the powder into a 10 mL test tube, added 12 drops of water, and inverted the tube until all the powder was saturated. We centrifuged each sample by tapping the test tube to the end of a 70 cm string tied to a small rock and spinning it until a distinct pellet formed at the bottom. We pipetted the supernatant onto the Brix Refractometer and read the approximate percent sugar from the Brix Table of Sugar Content of Aqueous Solutions. We compared the sugar contents of the three treatments using an ANOVA model that also included trees.

**Bark Microscopy**
We obtained a thin shaving of bark, placed it between a slide and coverslip, and examined the slide under a compound microscope at 100x magnification to look for chloroplasts.

**RESULTS**

**Brix Refractometry**
The sugar content of experimentally light-deprived samples was about 30% lower than that of either the sunny or the shady samples (Fig. 2). The $F$-test for differences among treatments was $F_{2,16}=2.95$, $P=0.08$. In post-hoc comparisons, the sugar content of the shaded treatment was statistically higher than that of the experimental treatment, but

![Figure 1: The regression line of the standard curve was used to calculate % sugar of sample solutions.](image1)

![Figure 2: The experimental light-deprived treatment had a lower sugar content than the sunny or shady treatments.](image2)
the sunny treatment, which had a slightly lower sugar content than the shaded treatment, was not separable from either shady or experimental treatments (Fig. 2). Trees were quite similar in sugar content ($F_{8,16}=1.53, P=0.22$). From our standard curve, the mean sugar content of the shaded, sunny, and experimental treatment supernatants was 0.85%, 0.81%, and 0.55% respectively.

**Light Microscopy**

We were unable to adequately visualize the cell contents in the green bark of *B. simaruba* and therefore could not determine whether chloroplasts were present.

**DISCUSSION**

The lower sugar content of the experimentally light-deprived treatment compared to that of the sunny and shady sides of trees suggested that photosynthesis occurs in the green bark cells of *B. simaruba*. However, we did not find evidence for differing rates of photosynthesis between the unmanipulated shady and sunny treatments. It is possible that all areas of the trunk received enough ambient sunlight for light to not be the limiting factor in the bark’s photosynthetic rate. If both the sunny and shady areas were performing photosynthesis at the cells’ peak rate, it would explain the lack of difference in sugar content. It is also possible that we did not select trunk sections that accurately represented sunny areas and shady areas. Due to our study site’s nearness to the equator ($10.3^\circ$ N latitude), there was not a conspicuous difference in light exposure between the north and south sides of the trunk. The rates of photosynthesis between the two site types may have been the same due to similar sun exposure between treatments.

*B. simaruba* undergoes a period of leaf senescence, apparently reducing its level of water loss by transpiration while simultaneously exposing its bark to sunlight by decreasing canopy cover. Its photosynthetic bark likely allows the tree to manufacture sugars during senescence instead of being fully dormant. To further understand the role of photosynthetic bark in *Bursera*’s success, it would be useful to examine water loss from the bark during photosynthesis compared to water loss through leaf stomata. Its bark may allow the tree to produce enough sugars to survive the dry season without the high water costs of keeping its leaves. We observed that *B. simaruba* trees often co-occurred with cacti in sunny, dry, rocky, and steep areas where fewer trees of other species seemed to grow, supporting the idea that its photosynthetic bark gives it a unique niche and may be a key innovation for minimizing water loss during the dry season while still accomplishing some photosynthesis. Examining sugar production in *Bursera* bark compared to leaves and across seasons would further clarify the role of the bark in *Bursera*’s success. It may be providing just enough sugar to allow the tree to survive the dry season and live in otherwise uninhabitable places, or it may be a primary source of carbohydrates for the plant.

Photosynthesis in the bark of *B. simaruba* may indicate a key evolutionary innovation that allowed the *Bursera* genus to diversify through adaptive radiation. The most crucial next step in investigating this clade is to determine whether photosynthetic bark is a basal trait of the *Bursera* genus. For a key innovation to be the cause of high diversity within a clade, it needs to be shared by all members of the clade. Identifying key innovations brings us closer to understanding the global distribution of species and biodiversity.

**ACKNOWLEDGEMENTS**

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**AUTHOR CONTRIBUTIONS**

“HNM created the figures. Authors contributed equally to other components.”

**LITERATURE CITED**


BEHAVIORAL MODIFICATION IN THE PRESENCE OF JUVENILES BY *Jacana spinosa*

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Faculty Editor: Matthew P. Ayres

Abstract: Across the animal kingdom, adults often change their behavior when caring for young. Behavioral changes may include investing additional time and energy into ensuring the success of juveniles. The division of labor within polyandrous societies is unusual in that males, rather than females, adopt the majority of parental care. We observed adult *Jacana spinosa*, a polyandrous species, in Palo Verde National Park, Costa Rica for three days to determine whether their behavior changed with the presence of juveniles. We found a clear dichotomy in the behavior of *J. spinosa* adults depending on the presence or absence of juveniles. Despite the sexual role reversal in *J. spinosa*, behavioral changes due to the presence of juveniles follow the general trend of increased attentiveness that exists across animal species.

Keywords: behavior, Northern Jacana, polyandry

INTRODUCTION

Across many taxa, the care of young animals can alter the behavior of the adults around them. From humans, who tend to shape their lives around their children, to cichlids, who raise their young in their mouths, animals that invest in parental care often exhibit costly behavior when raising young. This is consistent with Hamilton’s Rule (rB>C), which predicts that individuals will act in the interest of their relatives so long as the cost incurred (C) is less than the product of the benefit accrued (B) and the relatedness of organisms (r). However, in the construction of a polyandrous society, there can be questions of paternity such that males may not be related to the young that they raise, and yet still incur costs to support them. The question remains whether Hamilton’s rule applies in this rare sexual system, in which females reproduce with multiple males and contribute little to parental care.

The polyandrous society of Northern Jacanas (*Jacana spinosa*), in which females have a large territory occupied by one to four male mates, has been of particular interest to behaviorists since the 1970s (Betts and Jenni 1978, Betts and Jenni 1991). In this species, as per the sex role reversal implied in polyandry, males provide the majority of parental care to juveniles, and may change their behavior to accommodate young. For the two months required to raise their offspring, adults remain within five meters of chicks for 77% of daylight hours, and in 59% of that time, the adult is male (Forti and Nórbregz 2012, Betts and Jenni 1978). One study found that juveniles will forage faster around males than without them, so the presence of an adult may boost the juveniles’ survival (Betts and Jenni 1978). Females will sometimes help with parental duties, but usually only when helping the male protect the chicks from predators such as the Purple Gallinule (*Porphyrio martinicus*). If *J. spinosa* follow Hamilton’s Rule, individuals in the presence of juveniles (usually males) will perform very different behaviors from individuals free from the yoke of parental responsibility (typically females). For example, adults in the presence of juveniles could be expected to spend more time foraging for food, while adults without juveniles could be expected to devote more time to social interactions. However, if Hamilton’s Rule does not apply to *J. spinosa*, or if the social young of males are frequently not their genetic progeny, behaviors between adults with and without juveniles would likely not differ.

METHODS

We conducted research from 9 to 11 January 2017 in the marsh near the Tempisque River in Palo Verde National Park, Costa Rica. Our study area was the northeast region of the marsh visible from an elevated boardwalk at Palo Verde OTS Biological Research Station. Using a focal individual study with ten-minute observation periods, we observed the behavior and social interactions of adult *J. spinosa* with and without the presence of juvenile *J. spinosa* using Bushnell binoculars (8x42).
We did not use blinds because we assumed birds were relatively habituated to humans due to the frequently occupied boardwalk, as in Betts and Jenni 1991. The primary sampling periods were from 0700 to 1000 and 1400 to 1630. One individual adult may have been observed multiple times during the study, but never twice in a row or by two observers in one observation period to avoid sample bias. Only adult *J. spinosa* were observed, distinguishable by their darkly colored plumage.

We recorded the number of events in which focal birds displayed any of twelve different behaviors, or events, including the following: vocalization, antagonistic behavior towards a conspecific, wing-up, flight, snaking head, foraging, vigilance, hopping, walking, preening, loafing, and antagonistic behavior towards a heterospecific. We performed a chi-squared test to determine whether the frequency of different behavioral events differed between birds with and without young. We went on to classify the twelve behaviors into three separate operational categories: social (vocalization, wing-up, flight, snaking head, or antagonistic behavior towards a conspecific or a heterospecific), foraging (foraging, vigilance, hopping, or walking), and maintenance (preening or loafing). For each bird, we scored the total number of events that fell into each behavior type. We conducted t-tests to compare the mean number of social, foraging, and maintenance events, as well as total events, between individuals with and without juveniles.

We also used a Principle Components Analysis (PCA) to compare the behavior of *J. spinosa* adults with and without young. From this, we created a biplot comparing the behaviors of adults in the presence and absence of juveniles. We used t-tests to compare the behavior of adult Jacanas in the presence or absence of juveniles with respect to PC-1 and PC-2. All statistical analyses were conducted in JMP Pro 12.

**RESULTS**

In 57 10-minutes observation periods, we observed 39 individuals without juveniles and 18 with juveniles, recording a total of 371 behavioral events. Individuals with and without young differed in the relative frequency of some behaviors ($\chi^2 = 22.39$, df = 8, $P = 0.004$; Table 1). Birds without young compared to those with young were more likely to exhibit flights and wing-ups and less likely to exhibit vigilance and walking (Table 1).

<table>
<thead>
<tr>
<th>Event</th>
<th>No Young</th>
<th>Young</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foraging</td>
<td>0.32</td>
<td>0.34</td>
</tr>
<tr>
<td>Flight</td>
<td>0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>Vigilance</td>
<td>0.14</td>
<td>0.21</td>
</tr>
<tr>
<td>Wing Up</td>
<td>0.14</td>
<td>0.08</td>
</tr>
<tr>
<td>Vocalization</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Preening</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Antagonistic Behavior (conspecific)</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Hopping</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Walking</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Snaking Head</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Loafing</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Antagonistic Behavior (heterospecific)</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>394</td>
<td>158</td>
</tr>
</tbody>
</table>

There was no significant difference in the number of behavioral events belonging to different categories between individuals with and without juveniles present (social: $t_{44} = 1.48$, $P = 0.14$; foraging: $t_{41} = 0.72$, $P = 0.48$; maintenance: $t_{35} = 0.62$, $P = 0.54$; Figures 1b-d). Nor was there any difference in the total number of events performed...
by adults with and without juveniles ($t_{55} = 0.71, P = 0.48$; Figure 1a, $t_{55} = 1.35, P = 0.19$; Figure 2).

The first two axes of a Principle Components Analysis of behaviors explained 43% of the total variance in 12 behaviors (Table 2). The first principle component (PC-1) had positive loadings from almost all behaviors and so was interpretable as overall activity of adults (total number of behavior events). The second principle component (PC-2) had a mix of positive and negative loadings, and so represented different types of behaviors (Table 2). Adults with and without juveniles did not differ in terms of PC-1 but separated in terms of PC-2 (Figure 2). Thus, adults in the presence of juveniles were about equally active as adults without juveniles, but differed in their behavior types: more walking, vigilance, head snaking, and antagonistic behavior toward conspecifics. In contrast, adults without juveniles were more likely fly, display wing-ups, and engage in antagonistic behavior towards conspecifics.

**DISCUSSION**

Our results demonstrated behavioral differences between adults with and without juveniles. Individuals with juveniles displayed increased vigilance and walking, and individuals without juveniles engaged in more wing-ups and flight. This seems to follow the general trend of behavioral changes exhibited by animals with juveniles, adults sacrificing social interactions to protect the young and boost their offspring’s survival. Adult *J. spinosa* around juveniles (likely male) may be more vigilant to protect juveniles from predators, such as the Purple Gallinule, which live in close proximity to *J. spinosa* in the population we observed. The increased walking of these adults may be the result of walking with chicks while foraging, to protect them as well as to increase their foraging (Betts and Jenni 1978, 1991). The changes in behavior exhibited by the adults around the juveniles, therefore, seem to be devoted to the well-being of the young. In contrast, the behaviors of unaccompanied adults (likely female) seem to benefit themselves. Wing-ups are territorial displays, and the flights we observed often involved intraspecific interactions. These behaviors seem more social in nature, and are likely geared toward maintaining territories, which are crucial to female reproductive success.
Table 2. The eigenvectors for the first and second axes of the PCA.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>PC - 1</th>
<th>PC - 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vocalization</td>
<td>0.51</td>
<td>0</td>
</tr>
<tr>
<td>Antagonistic Behavior</td>
<td>0.32</td>
<td>-0.20</td>
</tr>
<tr>
<td>(conspecific)</td>
<td>0.48</td>
<td>-0.09</td>
</tr>
<tr>
<td>Wing-Up</td>
<td>0.24</td>
<td>0.34</td>
</tr>
<tr>
<td>Antagonistic Behavior</td>
<td>0.10</td>
<td>0.55</td>
</tr>
<tr>
<td>(heterospecific)</td>
<td>0.08</td>
<td>0.40</td>
</tr>
<tr>
<td>Flight</td>
<td>0.07</td>
<td>-0.08</td>
</tr>
<tr>
<td>Head Snaking</td>
<td>-0.02</td>
<td>0.33</td>
</tr>
<tr>
<td>Foraging</td>
<td>-0.16</td>
<td>-0.03</td>
</tr>
<tr>
<td>Vigilance</td>
<td>-0.07</td>
<td>0.45</td>
</tr>
<tr>
<td>Hopping</td>
<td>-0.26</td>
<td>0.17</td>
</tr>
<tr>
<td>Walking</td>
<td>-0.08</td>
<td>-0.13</td>
</tr>
<tr>
<td>Preening</td>
<td>-0.16</td>
<td>-0.03</td>
</tr>
<tr>
<td>Loafing</td>
<td>-0.07</td>
<td>0.45</td>
</tr>
<tr>
<td>Percent Variance (%)</td>
<td>27</td>
<td>16</td>
</tr>
<tr>
<td>Cumulative Variance (%)</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

Our operational categories did not capture behavioral differences between individuals with and without juveniles, even though other analyses revealed such differences.

The short time and small geographic area in which we collected our data limit the extent to which the results can be applied to *J. spinosa* as a species or polyandrous species in general. We were only able to observe individuals visible from the elevated boardwalk in the Palo Verde marsh, sampling a small portion of the birds present in the greater Palo Verde area. Additionally, because male and female *J. spinosa* are phenotypically similar and differ only in size, we were not able to distinguish sex during observation. Presumably, our comparisons of birds with and without young were frequently comparisons of males versus females, respectively (Betts and Jenni 1978). It would be helpful in future studies if it were possible to know the sex and individual identity of

![Figure 2. The biplot of PC 1 and PC 2 shows that adults with and without young did not differ with respect to PC 1 (t_{55} = 1.35, P = 0.19) but separated with respect to PC 2 (t_{55} = 1.73, P = 0.03).](image)
Hamilton’s rule \((rB>C)\) was consistent with our results despite the polyandrous nature of *J. spinosa*. In this case, males can forage more efficiently without juveniles but sacrifice their energy intake by allowing juveniles to accompany them while foraging. In theory, the male’s genetic relatedness and the benefit to the juvenile outweighs the cost of reduced energy intake. However, in a polyandrous society, females may have more than one male mate at a time, often two or three in the case of *J. spinosa*. Due to this potential lack of genetic relatedness (and thus smaller \(r\) value), the male may end up caring for offspring that are not his own. The female receives a greater reproductive benefit by engaging in extra pair copulations but does not have to invest much additional energy into the care and raising of her offspring. This asymmetry in initial investment in gametes by males and females (sperm versus eggs) may contribute to the fact that polyandry is a relatively rare reproductive system. However, the males in the observed *J. spinosa* population still followed the trend of behavioral alteration in order to care for juveniles. Still, it would not be surprising if Hamilton’s Rule is less predictive of behavior in a polyandrous society than in the more common cases of social monogamy and polygyny.

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We would like to thank the staff of Palo Verde Biological Station for their hospitality. We would also like to thank our TAs and fellow students for feedback and support, as well as Dr. Matt Ayres for aid in data analysis and refining our findings.

**AUTHOR CONTRIBUTIONS**

All authors contributed equally.

**LITERATURE CITED**


NEAR, FAR, WHEREVER YOU ARE: EFFECTS OF EXPOSED LIMESTONE ON TERRESTRIAL SNAIL SHELLS

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Abstract: Understanding resource limitations on biological processes can help explain population abundance and distribution. By extension, calcium from calcium carbonate (CaCO₃) in limestone deposits may affect the presence of CaCO₃-derived terrestrial snail shells. If snails are calcium limited in developing their shells, and limestone is the main source of calcium in the ecosystem, then being closer to limestone should permit more rapid snail growth, higher survivorship, and higher reproduction. We studied terrestrial snail populations at three limestone outcrops in a seasonally dry deciduous forest. At each outcrop, we measured the abundance, length, and relative thickness of each collected shell, categorized by morphospecies. The abundance of both morphospecies was significantly higher closer to the outcrops, but length and relative thickness of the two major shell morphospecies did not vary between near and far plots. Our study indicates that calcium may be a limiting resource for shell development and that increased calcium availability may allow snails to develop their shells more quickly even if the thickness is unaffected. These results contribute to understanding how less common forms of limiting resources may influence species abundance and distribution in the environment.

Key words: calcium carbonate, limestone, resource limitation, snail shells, snails

INTRODUCTION

All organisms are limited by the availability of one or more resources that directly affect an organism's ability to grow. One example is nitrogen limitation on net primary production in terrestrial ecosystems. Well-adapted plants tend to alter resource acquisition to maximize the capture of their most limiting resource. If plants are released from nitrogen limitation through the addition of fertilizer, growth increases. This increased growth rate could benefit an organism by reducing vulnerability to size-dependent predation, increasing access to other resources, and improving fitness by decreasing the time to reach reproductive viability.

Calcium is another nutrient that is crucial for some biological processes, including vertebrate bone growth. Similarly, terrestrial snails use calcium to build their shells, and for a variety of processes, including pH homeostasis, reproductive activity, cellular waste metabolism, and the formation of the epiphragm, a temporary seal used during hibernation (Dimitriadis 2001). Therefore calcium is a necessary macronutrient for terrestrial snails (Dallinger et al. 2001). Snails obtain calcium from their diet of fungi and detritus. The ultimate source of calcium in most terrestrial ecosystems is the parent material from which soil has been formed.

In Costa Rica, the parent material sometimes includes limestone, which is a legacy of ancient coral reefs that rose from the seafloor with the formation of the land bridge between North and South America (Wallace 1997). Along the Guayacán trail at Palo Verde National Park in Costa Rica, there are a series of exposed limestone outcrops that are surrounded by many scattered, intact snail shells. Limestone is mostly comprised of calcium carbonate (CaCO₃), the same mineral found in snail shells. If snails are calcium limited, and limestone augments calcium availability in the ecosystem, then being closer to the limestone should benefit the snails. Organisms that are released from resource limitation through shifts in nutrient cycling can be generally expected to grow faster and survive better. Therefore, snails near the exposed limestone were predicted to be larger, stronger, and more common than snails farther from the limestone. We tested this by comparing snail shells near and away from outcrops with respect to: number per area, length, and shell wall thickness.
Methods

Study site and sample collection
We collected snails from three outcrops of limestone in a seasonally dry deciduous forest along the Guayacán trail in Palo Verde National Park, Costa Rica. We collected data on 9 January 2017 (dry season) in clear, dry, sunny conditions. We chose outcrops to be accessible and generally similar, although the third outcrop was more vegetated than the other two. We attempted to collect all snail shells within two 10 m x 40 m plots at each outcrop. One plot was close to the exposed limestone rock and is henceforth referred to as “near,” while the other plot (“far”) was 10-15 m away from the edge of the near plot. All parts of all plots were searched by two people to minimize overlooked shells.

Data collection
We separated the shells into morphospecies (Figure A1). For each individual snail of each morphospecies we recorded the abundance of shells per 10 x 40 m plot. Using calipers, we measured the length of each shell from the apex to the top of the aperture and measured thickness at the bottom of the aperture (Figure A2).

Statistical analyses
Morphospecies A and B were common enough to permit statistical analyses. We tested for patterns in snail size and shell thickness with an ANOVA model that included outcrop (1-3) and plots (near or far). Analyses of shell thickness were based on the residuals of a regression of thickness versus shell length (Figures A3 and A4) to adjust for the pattern of increasing shell thickness with increasing shell length. We compared shell abundance of near versus far plots with a paired t-test (one pair of values for each of the 3 outcrops). Statistical analyses were conducted using JMP Pro 12 and Microsoft Excel.

Composition of outcrop rock and snail shell
To confirm the mineral composition of the outcrops, we collected small samples of rock for analysis. We weighed and then submerged a small rock fragment in 4-5% acetic acid vinegar solution to look for air bubbles expected from dissolving calcium carbonate into calcium acetate, water, and carbon dioxide (Blinkova & Eliseev 2005):

\[
\text{CaCO}_3 + \text{CH}_3\text{COOH} \rightarrow \text{Ca(C}_2\text{H}_3\text{O}_2)_2 + \text{H}_2\text{O} + \text{CO}_2
\]

After completion of the reaction, the rock sample was re-weighed to estimate the loss of calcium carbonate content. We followed the same procedure (but without measuring mass) with a shell sample to confirm the presence of calcium carbonate in snail shells.

Results
We collected a total of 239 snail shells representing five morphospecies, of which 55.6% were morphospecies A and 35.6% were morphospecies B. The remaining 8.8% of shells were: morphospecies C (4.6%), morphospecies D and E (1.7% each), and morphospecies F (0.8%) (Figure 1).

At all outcrops, there were relatively more shells of morphospecies A than of morphospecies B (Table 1). Relative abundance of morphospecies A and B did not differ greatly among outcrops (chi-square = 5.30, \( P = 0.07 \), df = 2), although morphospecies A tended to be relatively more abundant at outcrop 3.
Figure 2. Shell abundance, shell length, and relative thickness of morphospecies A at plots near and far from three limestone outcrops in Palo Verde National Park. Standard errors are indicated for length and thickness.

Figure 3. Shell abundance, shell length, and relative thickness of morphospecies B at plots near and far from three limestone outcrops in Palo Verde National Park. Standard errors are indicated for length and thickness.
Table 1. At each outcrop, morphospecies A comprised the majority of collected snails.

<table>
<thead>
<tr>
<th>Outcrop</th>
<th>Morphospecies A (% abundance)</th>
<th>Morphospecies B (% abundance)</th>
<th>Total shells per outcrop</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>44</td>
<td>126</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>36</td>
<td>61</td>
</tr>
<tr>
<td>3</td>
<td>77</td>
<td>23</td>
<td>31</td>
</tr>
</tbody>
</table>

**Morphospecies A**
Morphospecies A was more abundant at near plots than far plots (paired-\( t = 3.75\), one-tailed \( P = 0.03\), df = 2; Figure 2a). Shell length was similar between near and far plots (\( F_{2, 100} = 0.30\), \( P = 0.59\); Figure 2b), shell length varied among outcrops (\( F_{2, 100} = 9.25\), \( P = 0.0002\)), and there was no significant interaction between outcrop and plot (\( F_{2, 100} = 2.74\), \( P = 0.07\)). There were no significant effects of outcrop, plot, or their interaction on shell thickness (adjusted for effects of shell length) (Figure 2c). \( F_{2, 100} < 0.55 \) and \( P > 0.19 \) for all three tests.

**Morphospecies B**
Abundance of morphospecies B was higher between near and far plots (paired-\( t = 1.58\), one-tailed \( P = 0.13\), df = 2; Figure 3a). There was no evidence for effects of near versus far or outcrop in the length or relative shell thickness of morphospecies B (Figure 3b, Figure 3c). The absence of snails in the far plot at outcrop 3 precluded statistical tests with a two-way ANOVA.

**Limestone Chemical Data**
A 22.329 g rock sample from outcrop 1 was reduced to 20.758 g (7.04% dissolution) after being immersed in 100 ml of 4-5% acetic acid vinegar solution for 7.5 hours. The sample immediately bubbled upon contact with the vinegar, and we recorded its final mass after bubbling ceased. A sample shell immersed in the vinegar solution also produced bubbling upon contact.

**DISCUSSION**
The strikingly higher abundance of snail shells near limestone versus just 10-15 m away (significantly so for morphospecies A) supported the hypothesis that limestone deposits provide a limiting resource to snails. We did not see any snail shells farther from the outcrops than our far study plots. One possible mechanism is higher survival of snails where calcium was more abundant. Since calcium carbonate is the primary component of limestone deposits (Kiliç 2013), our abundance data support the idea that these deposits provide a source of calcium that is useful to snails. However, our length and relative thickness measurements across morphospecies A and B did not reveal effects of calcium availability on snail size or shell thickness. Shell thickness may be a developmentally regulated process in our snail populations. Thus, calcium may be a limiting resource for shell development that, when more accessible, allows snails to grow their shells more quickly even if thickness was invariant. Higher growth rates could lead to higher snail survivorship or earlier reproduction, resulting in the higher abundance of shells near outcrops.

While our results supported the hypothesis that calcium is a limiting resource for snails, snail growth and abundance may be further affected by other processes. Although immersing a sample of the outcrop rock in 4-5% acetic acid solution confirmed the presence of calcium carbonate, the weak pH of this available reagent was insufficient to determine whether the limestone is composed entirely of calcium carbonate. Furthermore, the relative abundance of morphospecies A at outcrop 3 seemed to be higher than at other outcrops, suggesting that another factor at that location was more favorable to morphospecies A than to morphospecies B. Perhaps other trace resources typically found within limestone, such as iron, sodium, or potassium (Kiliç 2013) further affect snail development and distribution. A greater abundance of shells near limestone deposits may also result from shell deposition by snail predators that prefer the relatively open clearings of the deposits. However, we observed several morphospecies B shells that were adhered to the underside of outcrop rock faces, indicating that these shells were not transported there postmortem by predators. Yet, the age and extent of decomposition of the shells were also unknown and may have limited our ability to effectively assess the distribution of live versus deceased snail populations. Regardless, the difference in shell...
abundance across plots, even within the 10 m difference between near and far plots, supports the hypothesis that the limestone deposits somehow support higher snail abundance. All outcrops were relatively consistent in vegetation cover between near and far plots. Thus, the high abundance of shells at near plots was not an artifact of lower shell visibility on the forest floor.

Further investigations could explore the life history and development of the snail morphospecies in relation to limestone outcrops. We were unable to find any living snails during our study period, which could be because these snail species hide and become inactive during the dry season to avoid desiccation (Cook 2001). If so, we would expect live snails to be evident near the outcrops during the wet season. Tracking the distribution and dispersal of these live populations could reveal where the morphospecies reproduce, lay eggs, and feed. Live observations could also reveal predation rates and distributions across plots, as well as predator behavior. Alternatively, mortality could be related to higher desiccation rates of snails on limestone outcrops than those on the forest floor. Finally, direct observations of the fungi species and detritus that the snails eat in both near and far plots could be matched with an analysis of the calcium content of those food sources and the associated soil. If samples indicate a high calcium content in consumed fungi or detritus of near plots, or if morphospecies appear to prefer laying eggs near limestone outcrops, this could help explain the greater abundance of shells near sources of calcium.

Apparently, terrestrial snail shells can be an indicator of the influence of a mineral resource on the local ecology. In accord with Liebig’s Law of the Minimum, snail growth must be limited not by the total amount of resources available, but rather by the scarcest of those resources. There are numerous examples of nitrogen and phosphorous limitation in terrestrial and marine ecosystems (Sterner & Elser 2002), but calcium is not frequently noted as a limiting resource. Our results suggest, however, that calcium is a limiting resource for the two major morphospecies of terrestrial snail found in Palo Verde. Some vertebrate predators such as birds rely on snails as a source of calcium (Taliaferro et al. 2001), which suggests that resource limitation on low trophic levels can have implications that reverberate up the trophic structure. Thus, this study contributes to understanding how less commonly limiting mineral resources can influence species abundance and distribution.

ACKNOWLEDGEMENTS
We would like to acknowledge Romelio Campos for his generous gift of vinegar for our chemical analysis.

LITERATURE CITED
APPENDIX

Site coordinates

Outcrop 1, near plot: 10.34841667°N, 85.34050000°W
Outcrop 1, far plot: 10.34845000°N, 85.34045000°W
Outcrop 2, near plot: 10.34873333°N, 85.34175000°W
Outcrop 2, far plot: 10.34875000°N, 85.34158333°W
Outcrop 3, near plot: 10.34871667°N, 85.34341667°W
Outcrop 3, far plot: 10.34881667°N, 85.34328333°W

Figure A1. Examples of morphospecies, A to F.

Figure A2. Schematic of length and thickness measurements. Length was measured from the apex to the top of the aperture. Thickness was measured at the base of the aperture.

Figure A3. Shell thickness as a function of length for morphospecies A. Residuals from the regression were analyzed as relative shell thickness.

Figure A4. Shell thickness as a function of length for morphospecies B. Residuals from the regression were analyzed as relative shell thickness.
THE IMITATION GAME: HOW SEX MIMICRY INFLUENCES REPRODUCTION IN BEGONIA INVOLUCRATA

AMBER R. AHRONIAN, FRANCESCA C. GOVERNALI, CAROLINE V. KOLLER, GABRIELA J. LOWRY, EMILY B. OKUN

Abstract: Species that engage in biomimicry can receive fitness benefits. Female flowers of Begonia involucrata mimic its male flowers, deceiving pollinators into visiting them even though they do not offer a reward in return. While male and female flowers are nearly identical, some subtle differences between male and female inflorescences could increase the efficacy of female deception, thereby increasing the fitness of the plant. We investigated relationships between the inflorescence morphology, flower sex, and seed set of B. involucrata. Inflorescence volume and flower density varied between males and females, although females of all densities had similar seed sets. Though seed data did not support the conclusion that female inflorescence morphology is adaptive, the strength and nature of traits related to female reproduction in B. involucrata nonetheless have the appearance of traits that have been shaped by natural selection.

Keywords: Begonia involucrata, deception, inflorescence, mimicry, pollinator

INTRODUCTION
Species that engage in biomimicry can receive fitness benefits. In Batesian mimicry, the mimicking species obtains a benefit from imitating a dangerous species, without incurring the physiological cost of producing its own defenses. For example, false coral snakes (Erythrolamprus spp.) have evolved similar coloration to the more poisonous coral snakes (Micrurus spp.) to deter predators. In Müllerian mimicry, several species converge on a single aposematic appearance to deter predators. Examples of mimicry among plants are less common but also occur. For instance, the female flowers of Begonia involucrata, a monoecious perennial herb with imperfect flowers, mimic its male flowers. The two sexes are barely distinguishable, except by the pink ovaries located underneath female flowers, and the slightly different appearance of the stamen and pistil. Not all imperfect monoecious plants contain similar male and female flowers. Corn (Z. maize) for example, has its female reproductive organs within the cob and its male reproductive organs in the tassels above. But unlike B. involucrata, corn is a wind-pollinated plant and therefore faces a different set of selection pressures. B. involucrata flowers must attract insect visitors, such as the honeybee (Trigona grandipennis) for pollination to occur. However, the female flowers of B. involucrata offer no reward to visitors; only the males offer pollen. In most animal-vector pollination systems, female flowers offer nectar, heat, medicinal secondary metabolites, resins, or other rewards to attract pollinators. Male B. involucrata flowers offer the reward of pollen but female flowers attract visitors through deception.

Because flowers usually occur in single-sex inflorescences, the proportion of male inflorescences in a population should, in theory, be higher than that of females to maintain pollinator visitation. It is often the case in plant populations that there are more male than female flowers, as pollen is less biologically expensive to produce than ovules. In a population of plants in which females do not offer a reward, having a higher proportion of male flowers is even more critical. Presumably, pollinators are less likely to visit flowers of the species if they have been deceived more frequently, i.e. have visited more female than male flowers.

While male and female flowers must be nearly identical to deceive pollinators, subtle morphological differences that emphasize female inflorescences could increase the efficacy of female deception. For example, female inflorescences could be positioned higher on the plant where they would be more conspicuous to aerial pollinators.
Also, plants could produce female inflorescences that contain more flowers, occupy a larger volume, or contain a higher density of flowers compared to male inflorescences. Ågren and Schemske (2000) found that bee pollinators were preferentially attracted to relatively large flowers.

If female inflorescences are more likely than expected to be the highest inflorescence or have different morphology from male inflorescences, these differences could be adaptive. We tested whether variation in the morphology and location of female inflorescences affected pollination success by testing for correlations with seed set, a proxy for pollination success.

METHODS

Flower Data
We collected data from 113 B. involucrata plants from 17 to 19 January 2017 along a single trail in Monte Verde Cloud Forest Reserve, Costa Rica. For each plant, we recorded the sex of the highest inflorescence, and for each inflorescence, we recorded the sex of its flowers, the number of flowers, the number of seeds, and its dimensions. In the seeded plants we encountered, we counted the number of seeds per inflorescence as a measure of pollination success. We evaluated histograms and performed a chi-square test to analyze the relationship between number of inflorescences per plant and sex ratio of inflorescences. We also used a chi-square test to test whether the highest inflorescences were more likely than by chance to be female. For all plants that included both sexes, we evaluated number of flowers per inflorescence, inflorescence volume, and density of flowers per dm$^3$ within inflorescences with a general model that included sex, plant, and sex x plant. We also compared the sexes with respect to inflorescence volume and flower density with paired t-tests. We used a chi-squared test to analyze the relationship between proportion of seeds to flowers and number of inflorescences on the plant. We used a linear regression to analyze the relationship between proportion of seeds to flowers and inflorescence density.

Visitation
To observe visitation by potential pollinators, we watched 30 plants from one meter away for approximately 15 minutes each, noting the number and identity of any visitors that interacted with the reproductive organs of the flowers. We also recorded the sex of any inflorescences that were visited, and the number of flowers visited within each inflorescence.

RESULTS

Flower Data
In the 113 plants studied, there were a total of 182 inflorescences and 2,549 flowers. The sex distribution of inflorescences in the entire group was 65% male, 27% female, and 8% mixed.

The majority of the plants in our data set (66%) had only one inflorescence (Fig. 1). Of all plants with a single inflorescence, 80% were male, 11% were mixed, and 9% were female. Of plants with two inflorescences, 65% were male, 27% were female, and 8% were mixed. The ratio of female to male inflorescences was significantly lower in plants with one inflorescence versus plants with two inflorescences (chi-square = 5.69, $P = 0.017$, df = 1).

In plants with more than one inflorescence, neither males nor females were more likely to be the highest inflorescence on a plant (chi-square = 2.58, $P = 0.11$, df = 1).

The mean number of flowers per inflorescence was very similar between males and females: mean ± SE = 9.6 ± 1.0 and 8.4 ± 1.9, respectively ($F_{1,39} = 1.25$, $P = 0.27$; Table 1).
Table 1. Results from ANOVAs of inflorescence morphology in *Begonia involucrata*.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>P</th>
<th>F</th>
<th>P</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant</td>
<td>16</td>
<td>0.77</td>
<td>0.71</td>
<td>3.05</td>
<td>0.002</td>
<td>1.64</td>
<td>0.10</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>1.25</td>
<td>0.27</td>
<td>48.56</td>
<td>&lt;0.0001</td>
<td>40.91</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plant x Sex</td>
<td>16</td>
<td>1.05</td>
<td>0.43</td>
<td>2.36</td>
<td>0.01</td>
<td>0.66</td>
<td>0.81</td>
</tr>
<tr>
<td>Error</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

However, female inflorescences occupied more volume ($F_{1,39} = 48.6, P < 0.0001$; Fig. 2), and therefore had a lower density of flowers per cubic decimeter ($F_{1,39} = 40.9, P < 0.0001$; Fig. 2). There was no variation among plants in number of flowers per inflorescence or density of inflorescences, but there was some variation among plants in volume of inflorescences ($F_{16,39} = 3.05, P = 0.002$; Table 1). There was some interaction between sex and plant ($F_{16,39} = 2.36, P = 0.01$; Table 1).

About 22% of all plants, and about 50% of all female inflorescences, had seeding clusters. There was no significant difference in ratio of seeds to female flowers and seeds (a measure of pollination success) between plants with one versus two female inflorescences (chi-square = 2.18, $P = 0.14$, df = 1). The density of female inflorescences was unrelated to the proportion of seeds to flowers (Fig. 3).

**Visitation**

All 20 visitations that we observed were on male flowers. Eleven visitors were flies, six were small beetles, two were wasps, one was a spider, and one was a bee. The majority of visitors were already on the flower when we began watching and were still on the same flower within the same inflorescence when we left. Almost all visited only one flower while we were watching. The notable exception was the one bee that visited seven male flowers. We were unable to capture visitors to identify them. However, a previous study on *B. involucrata* reported that its main pollinator was...
the honeybee (*T. grandipennis*; Ågren and Schemske 1991).

**DISCUSSION**

*B. involucrata* produces female inflorescences with a different morphology than male inflorescences; the number of flowers per inflorescence did not differ but female flowers were distributed across more volume. This difference may be an adaptation of females that is more likely to attract the attention of pollinators, or more simply because larger targets are more likely to be landed upon. This is plausible, as Ågren and Schemske (2000) found that bees (common *B. involucrata* pollinators) are attracted to larger flowers. An additional advantage of a less dense female cluster could be to increase genetic diversity of offspring because a single visitor will not be as likely to pollinate all of the flowers if they are spread across a large volume. These modifications to the imitation of male inflorescences may be necessary because female flowers offer neither nectar nor pollen as a reward, so they must pose as males, and desirable ones at that, to attract pollinators.

The lack of differences between sexes in flowers per inflorescence could be due to architectural or developmental constraints. The plant may only be able to support or invest in a certain number of flowers per inflorescence, no matter how beneficial it would be to have more flowers. Another possibility is that the female inflorescences have been optimized for pollination success with equal numbers of flowers per inflorescence.

Presumably the pollinators of *B. involucrata* perceive patches of inflorescences on a coarser scale than individual plants. If so, the optimal sex ratio for an individual plant would depend in part on the sex ratio of its neighbors. For example, if a plant’s nearest neighbors are male-biased, it would be more adaptive to have more female inflorescences than if neighbors are female-biased. We did not collect data on spatial effects in *B. involucrata* patches, because some of the nearest neighbors of study plants were inaccessible. However, Tran et al. (2004) found that female inflorescences were most likely not optimally distributed in a population based on nearest neighbor sex. Furthermore, we know of no mechanisms by which a plant could perceive the sex ratio of its neighbors.

Most *B. involucrata* plants had only a single inflorescence. Of these single inflorescence plants, 80% were male. As ovules are more expensive to produce, they are the limiting factor in reproduction. Pollen is comparatively cheap to produce. Thus it may be adaptive for smaller plants with fewer resources to invest preferentially in male flowers. There was further evidence of strategic investments in that the proportion of female inflorescences was higher in plants with two inflorescences than in plants with one inflorescence.

The hypothesis that the morphology of female inflorescences is adaptive was weakened by the result that the abundance of seeds, a proxy for pollination success, was unrelated to inflorescence morphology (Fig. 3).

On occasion we encountered mixed-sex inflorescences. Only a few inflorescences contained flowers of both sexes, and generally there was only one outlier within a cluster of the opposite sex. There are a few possible explanations for this phenomenon. It could be a developmental abnormality that does not benefit the plant. Alternatively, it could be an adaptation allowing for greater success of pollinator deception that not all plants have yet converged on. In theory, it would be potentially beneficial to have one or two male flowers mixed in with a mostly female inflorescence, because male flowers...
provide an actual pollen reward. Additionally, male flowers could act as a fail-safe to give the plant a higher chance of having gametes involved in fertilization, even when it invests in a risky female inflorescence.

It would be informative if future investigations could better evaluate seed production relative to sex ratios and floral morphology. More and better observations of pollinator behavior would also be valuable.

Apparently, *B. involucrata* display some adaptations in floral and inflorescence morphology, but are also subject to physiological constraints in flower production. Though they are neither Batesian nor Müllerian mimics, *B. involucrata*’s self-mimicry has been effective enough at deceiving pollinators to maintain a large population in the Monteverde cloud forest. Most mimicry in the animal kingdom serves as a predatory defense system rather than a manifestation of sexual selection. Sexual selection has different pressures that famously lead to surprising results. Mimicry in *B. involucrata*, like in many other instances of sexual selection, has led to novel evolutionary adaptations.

**ACKNOWLEDGEMENTS**

We would like to thank our professor, Matthew Ayres, as well as our graduate teaching assistants, Madilyn Gamble and Braden Elliott, for comments and criticism.

**LITERATURE CITED**


THE EFFECTS OF BROMELIAD SIZE AND DENSITY ON MACROINVERTEBRATE COMMUNITIES IN PHYTOTELMATA

CALLUM H. BACKSTROM AND SAMUEL R. GOCHMAN

Faculty Editor: Matthew Ayres

Abstract: Understanding the processes that structure communities in geographically isolated ecosystems is a central problem in ecology. We studied the communities of aquatic invertebrates that inhabit the phytotelmata of bromeliads in a cloud forest. If phytotelmata are islands of habitats, they could follow predictions from the theory of island biogeography: i.e., bromeliads that are aggregated or have higher water volume should have more taxonomic richness than isolated or smaller bromeliads. We collected the contents of phytotelmata from thirty bromeliad plants to analyze water volume and abundance of different taxa and functional groups. Neither abundance nor taxonomic richness varied between aggregated and isolated bromeliad plants, yet there was some evidence of structure in the relative abundances of filterer and gatherer functional groups. Our results indicate that neither island biogeography nor niche theory are adequate to fully understand the communities that inhabit the isolated patches of aquatic habitat within bromeliad tanks.

Key words: bromeliads, macroinvertebrates, niche theory, phytotelmata, theory of island biogeography

INTRODUCTION

Diversity and coexistence within ecological communities depend on the environment. For example, relative geographic location can affect the diversity of organisms found in a given ecosystem. Three notable theories that have been proposed to explain the community structure are the theory of island biogeography, niche theory, and the neutral theory. The theory of island biogeography (MacArthur and Wilson 1967) predicts that the diversity of taxa in islands of habitat patches will be a function of island size and proximity to potential sources of colonizing organisms. Communities on islands that are closer to other source populations (i.e., other islands or a mainland region) or larger in size are predicted to have higher species diversity. Close proximity to other islands increases the chance that new species will immigrate to a given island, while larger island space reduces the risk of local extinction from predation, interspecific competition, or stochastic factors. Conversely, more isolated or smaller islands are predicted to have lower species diversity due to lower immigration rates and higher susceptibility to extinctions.

Niche theory provides another theoretical source of structure in biological communities. Under this model, taxonomic diversity depends upon the extent to which different species have diversified with respect to resource use, habitat selection, and predator avoidance among other things. Thus, one would expect more diverse communities to contain taxa with greater diversity in, for example, feeding methods, such as predation, gathering, or filtration.

Finally, in contrast to both the concepts of island biogeography or niche partitioning, there is the possibility that communities are better understood as random assemblages than as structured communities. According to the neutral theory (Hubbell 2001), species diversity within communities is maintained largely by chance rather than in the predictable ways that arise from island biogeography or niche theory.

One system that may be characterized as a set of isolated communities (islands) is that which occurs within the phytotelmata of bromeliads in cloud forests. Bromeliads typically occur as epiphytes on tree trunks and branches, where they collect water and organic debris in a rosette of bracts formed by leaves.
(Zuchowski 2005). The central tank formed by the tightly overlapping bracts is a phytotelma. When the phytotelma of a bromeliad accumulates water and leaf detritus, it may host a community of macroinvertebrates (Zuchowski 2005). Like literal islands, bromeliads can be large or small and occur in aggregations or isolation (Zuchowski 2005).

In the botanical garden of the Monteverde Biological Station in Costa Rica, there is a large population of bromeliads that are on or near the ground and therefore are more accessible than those in the canopies. Some are clustered together while others are relatively isolated. Both the clustered and isolated bromeliads vary in size and thus phytotelma tank volume.

If phytotelma communities are structured under the theory of island biogeography, then proximity to other plants and phytotelma size would affect the taxonomic richness and abundance of organisms. Therefore, bromeliads that are clustered together or have a higher phytotelma water volume would be predicted to have a higher number of taxa than isolated or smaller bromeliads. Phytotelma communities could also be structured by interspecific interactions (niche theory). This could be reflected in the relative abundance of different functional groups (filterers, gatherers, predators). If so, the relative abundances of different functional groups could be expected to be relatively stable among bromeliads. In contrast to predictions from niche theory, phytotelma communities could instead be random assemblages drawn from the regional pool of potential inhabitants. If so, there would be no pattern in community composition including in the relative abundance of different functional groups. We tested these alternative models with a study of the communities inhabiting the phytotelma of bromeliads in the cloud forest of Costa Rica.

METHODS

Study site and sample collection
We sampled phytotelma of bromeliads from the garden at the Monteverde Biological Station in Costa Rica on 18 January 2017 in clear, dry conditions. We sampled bromeliads that were in a dense aggregation (clustered) or were in relative isolation in the garden (isolated). Of the 80 plants in the cluster, we chose 20 for sampling using a random number generator; we were also able to locate and sample 10 isolated plants. All of the bromeliads that we sampled contained water and thus provided suitable environments for freshwater macroinvertebrates. For each of the 30 study plants, we extracted the entire liquid contents of the phytotelma with a large diameter pipette, transferred the contents to large vials, brought them to the laboratory for examination under dissecting microscopes.

Data collection
We measured the diameter of each phytotelma at the top of the tank and recorded total water volume of each sample. We then determined the number and taxa (often families) of macroinvertebrates in each sample and classified them by functional feeding group: filterer, gatherer, or predator.

Statistical analyses
Analysis of taxonomic groups
All abundance data were square-root transformed to improve normality before analyses. We performed a linear regression to describe the relationship between overall macroinvertebrate abundance and total water volume. We then compared AICc scores from this simple model (single regression function for all bromeliads) with more complex models that allowed for different intercepts, slopes, or both for isolated versus clustered bromeliads. We conducted the same analysis for number of taxa (i.e., taxonomic richness) per bromeliad.

To evaluate the variance in abundance among bromeliad communities, we calculated a dispersion coefficient (variance/mean) for the abundance of each taxon across bromeliads. A dispersion coefficient of 1 indicates random dispersion, while higher values indicate overdispersion and lower values indicate a tendency for uniform dispersion, which would be a signal of structure in macroinvertebrate communities.

We also compared the taxonomic composition of clustered and isolated communities with a principle components analysis (PCA). Specifically, we compared
clustered versus isolated communities with respect to the first two axes from the PCA.

Analysis of functional groups
We evaluated patterns in the abundances of functional feeding groups with a general linear model that included functional feeding group (filterer, gatherer, or predator), isolation condition (clustered or isolated), functional group x isolation, and water volume as a continuous variable.

We analyzed the variance of macroinvertebrate abundance to determine if the communities were different than expected by chance (i.e., structured or overdispersed in variance) by performing a randomization test in R version 3.3.2. Due to low predator abundance, we excluded this functional group from further analyses. Our randomization test evaluated variance in the ratio of abundances of filterers versus gatherers. We compared the observed variance in our data to the frequency distribution of such variances calculated from 1,000 simulations in which individuals were randomly allocated to simulated bromeliads such that: (1) the total proportion of filterers to gatherers matched our data, (2) the number of simulated bromeliads matched our data, and (3) the total number of animals per bromeliad matched our data.

RESULTS
Analysis of taxonomic groups
We recorded seven taxa within the phytotelmata of 30 bromeliads: Culicidae (mosquito larvae and pupae), Cyclopidae (copepods), Chironomidae (midge larvae), Psychodidae (fly larvae), Dityscidae (beetle larvae), Ceratopogonidae (fly larvae), and Oribatida (mites). We found strong linear relationships between water volume and both the total abundance of animals and taxonomic richness (Figure 1). Allowing for independent slopes among clustered and isolated plants did not increase the information content relative to using water volume alone (ΔAICc = 0.56 and 2.05 for abundance and taxonomic richness, respectively).

Table 1. Characteristics of macroinvertebrate taxa found in bromeliad tanks near the Monteverde Biological Station.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Dispersion coefficient</th>
<th>Functional group</th>
<th>Mean abundance</th>
<th>Variance of abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chironomidae</td>
<td>1.09</td>
<td>filterer</td>
<td>0.83</td>
<td>1.94</td>
</tr>
<tr>
<td>Culicidae</td>
<td>2.14</td>
<td>filterer</td>
<td>3.90</td>
<td>98.23</td>
</tr>
<tr>
<td>Cyclopidae</td>
<td>2.29</td>
<td>filterer</td>
<td>3.90</td>
<td>42.51</td>
</tr>
<tr>
<td>Oribatida</td>
<td>2.59</td>
<td>gatherer</td>
<td>0.37</td>
<td>3.34</td>
</tr>
<tr>
<td>Psychodidae</td>
<td>1.30</td>
<td>gatherer</td>
<td>1.17</td>
<td>4.07</td>
</tr>
<tr>
<td>Ceratopogonidae</td>
<td>1.15</td>
<td>gatherer</td>
<td>0.20</td>
<td>0.37</td>
</tr>
<tr>
<td>Dityscidae</td>
<td>1.43</td>
<td>predator</td>
<td>0.40</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Dispersion coefficients (variance-to-mean ratios) for all taxa were greater than 1 (Table 1). Those for Chironomidae, Psychodidae, Dityscidae, and Ceratopogonidae were close to 1, indicating near-random dispersion, while those for Culicidae, Cyclopidae, and Oribatida were considerably greater than 1, indicating clumping in their abundances among bromeliads.
Table 2. Correlation matrix for abundance of taxa (square-root transformed) within bromeliads.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Culicidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Cyclopidae</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Chironomidae</td>
<td>0.39</td>
<td>-0.20</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>D. Psychodidae</td>
<td>-0.07</td>
<td>-0.29</td>
<td>0.33</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>E. Dityscidae</td>
<td>0.06</td>
<td>-0.03</td>
<td>0.04</td>
<td>-0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. Ceratopogonidae</td>
<td>-0.24</td>
<td>-0.27</td>
<td>-0.05</td>
<td>-0.11</td>
<td>-0.16</td>
<td></td>
</tr>
<tr>
<td>G. Oribatida</td>
<td>0.18</td>
<td>0.36</td>
<td>0.07</td>
<td>-0.17</td>
<td>-0.10</td>
<td>-0.09</td>
</tr>
</tbody>
</table>

Across bromeliads, there were positive correlations in abundances between Culicidae and Chironomidae, Cyclopidae and Oribatida, and Chironomidae and Psychodidae; there were negative correlations between abundances of Cyclopidae and Psychodidae, Cyclopidae and Ceratopogonidae, and Culicidae and Ceratopogonidae (Table 2). The first principle component axis (PC-1) had positive loadings from Culicidae, Cyclopidae, Chironomidae, Dityscidae, and Oribatida; and negative loadings from Psychodidae and Ceratopogonidae (Table 3). The second principle component axis (PC-2) had positive loadings from Culicidae, Chironomidae, Psychodidae, and Dityscidae; and negative loadings from Cyclopidae, Ceratopogonidae, and Oribatida. The first two principle component axes explained 47% of the total variation. Isolated and clustered bromeliads did not differ along PC-1 ($F_{1,28} = 1.96$, $P = 0.85$) or PC-2 ($F_{1,28} = 0.90$, $P = 0.40$) (Figure 2).

Table 3. Loadings from a principle components analysis of the abundance of 7 taxa that occurred in the phytotelmata of bromeliads.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>PC-1 Loadings</th>
<th>PC-2 Loadings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culicidae</td>
<td>0.45</td>
<td>0.41</td>
</tr>
<tr>
<td>Cyclopidae</td>
<td>0.59</td>
<td>-0.21</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>0.02</td>
<td>0.67</td>
</tr>
<tr>
<td>Psychodidae</td>
<td>-0.30</td>
<td>0.50</td>
</tr>
<tr>
<td>Dityscidae</td>
<td>0.04</td>
<td>0.14</td>
</tr>
<tr>
<td>Ceratopogonidae</td>
<td>-0.36</td>
<td>-0.26</td>
</tr>
<tr>
<td>Oribatida</td>
<td>0.47</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

% Variance 25 22

Analysis of Functional Groups
The most abundant functional group was filterers followed by gatherers and then predators (Figure 3; $F_{2,83} = 17.95$, $P < 0.001$). There were no effects of isolation or functional group x isolation ($F_{1,83} = 0.03$, $P = 0.86$; $F_{2,83} = 1.88$, $P = 0.16$, respectively). Abundance was positively related to water volume ($F_{1,83} = 17.01$, $P < 0.001$).
DISCUSSION

Our results produced lines of evidence both supporting and refuting community patterns predicted by island biogeography. As predicted, higher-volume bromeliads had higher overall abundances and more taxa. However, there was no indication of differences between clustered and isolated plants, either in abundances, taxonomic richness, or taxonomic structure as characterized by principle component analyses. Thus, the theory of island biogeography explained some but not all properties of the communities within bromeliad “islands.”

There was some evidence of community structure from analyses of functional groups, particularly in filterers and gatherers. As indicated by the randomization test, our communities seemed to be comprised of either very low filterer abundance and high gatherer abundance, or vice versa. That is, the relative abundance of filterers and gatherers was nonrandomly irregular across phytotelmata. Apparently, the relative abundance of filterers and gatherers is nonrandom, but it is unpredictable which functional group will dominate. This could be a result of competition in which one group tends to outcompete the other but the outcome depends on individual abundances (Begon et al. 1990). Or, it could be that different phytotelmata vary in their basal resources (e.g., detritus), which enables either filterers or gatherers to dominate. Either of these mechanisms is consistent with niche partitioning. On the other hand, no other analyses revealed signals that were readily interpretable in terms of niche theory.

Neither island biogeography nor niche theory provided fully satisfying descriptions of the communities within bromeliad tanks. The high variance in filterer-to-gatherer ratio across bromeliad communities could be due to priority effects in which the first taxa to lays its eggs in a phytotelma will dictate the dominating functional group (filterer or gatherer) in that community. Culicidae, Cyclopidae, and Oribatida had much higher variabiity in their populations across plants than the other taxa. The high variance in the number of Culicidae across communities was partly due to a very high abundance from one bromeliad (n = 53), which was comparable to the abundance of culicids from all other bromeliads combined (n = 64). The variance in cyclopids and oribatids may be partly explained by dispersal limitations. While the other macroinvertebrate taxa have flying adults, adult oribatids must attach
themselves (phoresy) to other invertebrates to travel to new plants; cyclopid eggs are thought to disperse passively by attaching to aquatic insects and in wind-blown dust (Pennak 1953). Thus, the theory of island biogeography may be more applicable to cyclopids and oribatids than to the more mobile taxa.

Further investigations could explore the potential effects of competition between filterers and gatherers as well as the influence of island biogeography on a larger scale. Studying the interactions between filterers and gatherers in controlled, algae- or detritus-limited environments could indicate whether direct food source competition explains our observed results. This analysis could also reveal whether filterer or gatherer size has an effect on competitive ability, although we did not observe any apparent or consistent differences in the average sizes of individual macroinvertebrates within different functional groups. Moreover, our study was limited to one aggregation of bromeliads and nearby isolated bromeliads in the Monteverde Biological Station garden. Our “isolated” bromeliads were relatively close to the main cluster, so even the farthest distances between plants may not have limited flying adults. Future studies could analyze a greater number of isolated bromeliads farther from aggregations. This might reveal patterns of island biogeography on a larger scale.

Apparently, neither the island biogeography nor niche theory are sufficient alone to characterize the communities within bromeliad tanks. Probably, an understanding of bromeliad community structure requires a more nuanced consideration of principles from multiple models, including the idea that much variation is simply due to chance (neutral theory). Other important factors could include variability in basal resources and priority effects.

ACKNOWLEDGEMENTS
We thank the Monteverde Biological Station for allowing us to use the garden and laboratory for our study.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
COLLECTIVE DECISION-MAKING IN WHITE-NOSED COATIS (*Nasua narica*)

LOUISE J. BARIAS, REBECCA W. FLOWERS, AND MARIKO C. WHITENACK

Faculty Editor: Matthew P. Ayres

Abstract: Social animals often have to make decisions in novel situations that present uncertainty. The variably social white-nosed coatis (*Nasua narica*) may respond to uncertainty surrounding a foraging decision differently when they are solitary or part of a band. If coatis in a band make collective decisions, then their deliberation time for a foraging decision should tend to be longer than that of solitary coatis. In Monteverde, Costa Rica, we presented white-nosed coatis with six experimental trials of high and low risk foraging options. We measured the deliberation time at both low and high-risk sites and recorded the age and sex of the principle investigator. We found no significant effects of novelty, band size, or sex on deliberation time. However, adults were significantly more likely than juveniles to be the principle investigator for both low and high-risk sites. Our results suggest that coatis do not make collective decisions when in groups. Instead, it seems that foraging decisions are individualistic, even when within groups.

Key words: collective decision-making, *Nasua narica*, novelty, uncertainty, white-nosed coati

INTRODUCTION

Social animals often have to make decisions under uncertainty; that is, where there is a lack of information on the matter of consideration. For social animals living in groups, making good decisions on places to feed or nest benefits the individual fitness of group members. Groups may be better able to make good decisions because several decision-makers can eliminate individual error (Conradt 2012). However, groups must also resolve individual preference conflicts before making a final decision. Therefore, the process of making a decision for animals in groups could take longer. However, social animals living in groups can also act independently on decisions, depending on individual interests. In certain situations, such as when there is a limited amount of a resource, individuals in a group may compete against one another for the resource.

We tested with white-nosed coatis (*Nasua narica*) whether groups or solitary animals made decisions faster when presented with novel items while foraging. White-nosed coatis are variably social animals that can live as individuals (usually males) or in bands that range from 4 to 64 adult females and juveniles. We offered to free-ranging coatis a sequence of pairs of foraging opportunities, one of which was designed to be novel for these animals, and therefore to challenge their decision-making when presented with uncertainty. If foraging decisions by groups requires reconciliation of interests among group members, then their deliberation time before exploring novel and potentially risky foraging opportunities will be longer. This would not be true if decision-making is individualistic even when animals are in groups.

METHODS

Experimental Setup

We observed white-nosed coatis from 18 to 20 January 2017 behind the Monteverde Biological Field Station. In this time, we conducted six observation periods from 1000 to 1200, 1300 to 1500, and 1500 to 1700. During each observation period, we conducted matching experimental trials simultaneously at two locations. The two locations were intended to improve chances of observing both solitary coatis and coatis in bands. For the first two trials, one site was in the forest where the coatis were known to nest, and another was in a clearing behind the research station. For trials three through six, we changed the second trial site to a slightly forested area near the clearing. Each trial had two perspectives, either two observers or an observer and a game camera.

On each of six occasions, at each of our two test sites, we set out two foraging options for coatis that were designed to be novel or not (high risk or low risk). Each option was baited with food from the research station kitchen. The food was distributed in equal amounts for each trial. Novel, high-risk options were intended to present greater uncertainty associated with the food. Low risk
sites provided information about the food (usually because it was a pile of food on the ground, not covered by or in an object). For example, coatis could choose between eating food in a plastic bucket (high risk) or next to a plastic bucket (low risk). Other sources of novelty or risk were: buried food, funnels, nets, metal rods, and a decoy snake. We switched positions of the high and low risk sites with each trial to prevent the coatis from becoming habituated to either. The trials lasted a maximum of two hours, or until all the food had been eaten.

Observers recorded “deliberation time” for the first coatis, or “principal investigators,” to approach each site. Separate deliberation times were recorded at the high and low risk sites, whether the same coati investigated both or not. Also, if a solitary individual approached either of the sites after another solitary individual had eaten and left, both were recorded as principal investigators because they did not overlap and presumably learned nothing from the other coati’s behaviors.

Deliberation time was defined as the time from when the principal investigator was within one meter of a high or low risk site and when it began to eat the food, or left the one-meter radius. Observers also recorded the sex and age of the principal investigator(s), and whether they were solitary or in a band. The observations collected in the site with one observer were later verified with the game camera. Observers also took behavioral notes during observation periods.

Statistical Analysis
We applied a square root transformation to deliberation times to improve normality. We tested for patterns in deliberation time with respect to band size (band or solitary) and risk (low or high), and with respect to sex and risk with two-way ANOVAs. We also compared variance of deliberation time in high and low risk sites with a paired t-test (one pair of points from each of the 6 trials). Additionally, we analyzed footage from the game camera to determine the ratio of adults to juveniles in the study area; this permitted a chi-square test of whether adults and juveniles were equally likely to investigate experimental foraging opportunities. We conducted analyses in JMP 13 and Microsoft Excel.

RESULTS
In every trial, there were 2-5 coati interactions with both low and high-risk sites. We measured deliberation time for 39 white-nosed coatis. Of those, there were 29 solitary coatis and 10 coatis in

![Figure 1](image-url)

**Figure 1.** a) Deliberation time did not differ between bands and solitary white-nosed coatis at low or high-risk sites. b) Deliberation time did not differ between male and female white-nosed coatis at low or high-risk sites.
bands. There were no significant effects of band size, risk, or their interaction on deliberation time (Figure 1a). For all three tests, F_{1,16} < 0.72 and P > 0.41.

The principal investigators included 32 adults and 4 juveniles. We were unable to identify the age of the remaining 5 principal investigators, so we did not use those data points in our age analysis. We estimated a ratio of 115 adults and 90 juveniles in the population based on our game camera footage. We compared this ratio with the age distribution of principal investigators (32 and 4) and found that adults were significantly more likely than juveniles to be the first to approach our experimental setup (chi-square = 15.7, P < 0.001, df = 1).

We were unable to identify the sex of four coatis, so we excluded those data points in our sex analysis. Of the remaining 35 principle investigators, 21 were male and 14 were female. There were no significant effects of sex, risk, or their interaction on deliberation time (Figure 1b). For all three tests, F_{1,15} < 1.46 and P > 0.25.

When we pooled all observations across band size, age, and sex, we found deliberation time was similar at low and high-risk sites (paired-t = 0.05, P = 0.96, df = 5; Figure 2). However, we observed 24 principal investigators at low risk sites and 15 at high-risk sites.

**Behavioral Observations**

We observed bands of coatis ranging in size from 5 to about 40 individuals. Coatis varied their levels of investigation around our experimental setup. Frequently, coatis would approach a risk site, circle it at least once while looking and sniffing at it, and only then begin to eat. During the bucket trial, one coati ate from the low risk site, walked to the high-risk site, circled the bucket and peered over its edge, then walked away without eating from the bucket. Other coatis ate from both high and low risk sites within the same trial period for nets and funnels. During the net trial, coatis used their front paws to lift and move the net off of the food after circling and sniffing the high-risk site.

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**DISCUSSION**

Contrary to the hypothesis that groups make collective decisions, deliberation times were unaffected by band size. This could be due to intragroup competition. Coatis in bands forage together, which increases their chances of finding food, but also decreases the probability that any one individual will consume food that the band finds (Figure 1). It follows that when the band finds food, coatis compete with each other for this limited resource. This could explain the similarity in deliberation time between coatis in bands and solitary coatis. As females and juveniles tend to live in bands, and males tend to be solitary, the
similarity in deliberation time between sexes could be due to the similarity in deliberation time between bands and solitary coatis.

The tradeoff between finds per time and competition also suggests an optimal group size for coatis, where individuals could receive greater rewards due to less competition and more finds per time. We observed either solitary coatis or coatis in groups of five or more, suggesting that this may be the optimal group size. According to Pérez-Irineo (2016), the band size of white-nosed coatis in the Southeastern Mexican Rainforest averaged 9.0 ± 0.5 individuals. Perhaps this is the optimal group size on which coatis have converged, but it may depend on more factors than foraging, such as predation protection. A study of ring-tailed coatis by Hirsch (2011a) found that juveniles tended to cluster on the front edge of a group, poised for foraging, while adult females excluded juveniles from the center of the group, where they were most protected from predators. Living in a band of coatis would then be beneficial for the adult females for more than just foraging.

Optimal group size for foraging may be nearly inconsequential for populations of animals that are not limited by food. Hirsch (2011b) found that while individuals in the front of the band had greater foraging success when feeding on an easily exhaustible resource, like fruit, coatis were equally successful at foraging when feeding on an abundant resource, such as ground litter invertebrates. In the coati population we studied, there may have been enough food within their territory (a compost dump filled weekly) that every coati was able to eat as much as they wanted. In this case, neither number of finds per time nor competition were important factors in foraging. Therefore, optimal group size for coatis in our study population could depend on factors other than foraging.

Another potential explanation for our results may be an error in our experimental setup. If coatis usually approached food that was nearer to wherever they emerged from the forest, they may have spent more time investigating the risk site they approached first, regardless of risk level. The time investigating the next risk site (if it is the same coati, as was the case in several observations) might then be less because they recognize that there is food at the second pile. The investigation of the first risk site they saw as compared to the second could have confounded our deliberation time measurements across trials. Additionally, as we put food out twice a day over four days (including a pilot study), the coatis may have become habituated to food appearing at our test sites. This could have possibly decreased their deliberation time in either risk situation as the trials went on, despite the novel situation in each trial.

Adults were significantly more likely than juveniles to be the principal investigator. Juveniles may be more cautious than adults to approach risk situations, and only do so when imitating parents who have already approached. This may boost their survival by helping them to avoid potential dangers. Juvenile aggression towards adults may indicate a matrilineal dominance hierarchy within the coati population, such that a juvenile whose mother is more dominant than another adult female may have the authority to chase the less dominant adult female away from a pile of food. This potential dominance structure could explain why adult females are the principal investigators of the food, especially if the most dominant adult females are those that eat first, as they would have authority over other adult females and all the juveniles. In future studies, banding and identifying individuals and determining the genetic structure of the group might clarify the effect of dominance hierarchies in populations of white-nosed coatis.

Overall, deliberation time did not vary across groups or sex in investigations of uncertainty in this population of white-nosed coatis. This suggests that this population does not follow the social decision-making model when foraging. Instead, intragroup competition over the food we used to bait our experimental setup may have motivated individuals to act in their own best interests regardless of band size. We did not find evidence for social decision-making around novelty associated with food, but we do not reject the possibility that they make collective decisions in other situations.

ACKNOWLEDGEMENTS
We would like to thank Eylln from Monteverde Biological Station for sharing his knowledge about the local coati population and the kitchen staff for graciously allowing us to use their compost to bait our experimental sites.
LITERATURE CITED


A RISING DAM LIFTS ALL INVERTEBRATES: NIXING THE NICHE

LEAH VALDES, HANNAH NASH, AND ERIC BRUNNER

Faculty Editor: Matthew P. Ayres

Abstract: Numerous hypotheses have been proposed to explain the biodiversity of life on Earth. Particularly prominent are the competing viewpoints of niche partitioning and neutral theory. Niche theory posits that limited resources will select for species that can minimize competition by inhabiting specific, minimally overlapping niches. Such selection would produce greater biodiversity as a consequence of specialization. Alternatively, neutral theory proposes that chance plays a major role in determining species assemblages. We tested these theories with an investigation of stream macroinvertebrates in Monteverde, Costa Rica. We used natural dams, which caused significant changes in water flow and sub-surface topography, as environmental features expected to create different niches. We sampled benthic macroinvertebrates above and below eight dams, identified the organisms to functional group, and compared the species assemblages above and below dams. If niche partitioning applies, macroinvertebrates above and below dams were predicted to differ, while if neutral theory better explains species distributions, the assemblages should have been similar. Although there was a greater total abundance of macroinvertebrates above dams than below, the proportional representation of different functional groups was very similar above and below dams. To our surprise, results were more consistent with neutral theory than niche theory.

Key words: niche theory, neutral theory, community structure, macroinvertebrates

INTRODUCTION
Numerous hypotheses have sought to explain the biodiversity of life, but exactly how millions of species can co-occur on Planet Earth is still one of biology’s great mysteries. Niche theory and neutral theory both attempt to explain biodiversity within the context of habitat structure. Niche theory emphasizes the unique ecology of species to explain how multiple species coexist in the same community. Under this idea, competition for resources and avoiding predation are two prominent forces that select for specialization, with the outcome being that more species can coexist in one environment. Under niche theory, the foraging strategies that succeed best are expected to differ in different environments. Therefore, one expects that species composition changes between habitats.

Alternatively, neutral theory emphasizes the similarities among species, and argues for the importance of chance in which species are represented in a community. Such a community would be less sensitive to differences among environments; instead, communities would be better described as random assemblages drawn from a regional pool of species that might occur there.

Stream macroinvertebrates provide an opportunity to study the effects of environment on community structure. Stream invertebrates can be classified into different functional groups based on how and what they eat; the most common functional groups are shredders, scrapers, collector-gatherers, filter feeders, and predators. If these communities follow niche theory, the proportions of such functional groups will change as streams change. However, if they are approximately random assemblages as neutral theory predicts, variation in stream environments will have relatively little effect on the relative abundance of different functional groups.

To evaluate these competing theories, we examined stream macroinvertebrates in Monteverde, Costa Rica. We tested whether differences in the stream’s physical environment, created by natural dams, would result in differences in macroinvertebrate community structure. The environments above dams have slower moving water, smaller substrate particle size, and higher concentrations of allochthonous resources, while the environments below dams have faster moving water, larger substrate particle size, smaller concentrations of organic matter, and higher concentrations of dissolved oxygen. If niche theory applies, one would expect different proportions of functional feeding groups above and below the dams. Under neutral theory, these
environmental differences would have little or no effect on invertebrate assemblages.

METHODS

Stream Sampling
On 18-19 January 2017, we sampled dams at eight locations along a stream in the cloud forest of Monteverde, Costa Rica. Dams were identified as sub-surface features such as rocks or logs that generated a slower current upstream and whitewater directly downstream. We chose the eight locations based on accessibility and quality. We chose dams that were near the access trail, had spots for two kick sample replicates above and below, and had substrate that was suitable for kick sampling. At each location, we took two kick samples above and below the dam. For each kick sample, a D-net was placed on the surface of the benthos and sediment was disturbed from within half a meter upstream of the net for 30 seconds. The contents of the net after sampling were collected in a plastic bag, labeled with dam number, position and replicate, and brought back to the lab. When large crustaceans were encountered (four in total), they were photographed, recorded, and released back into the stream. We measured dissolved oxygen and temperature above and below each dam using a YSI multimeter. We recorded a waypoint with a GPS at each dam.

Data Analysis
In the lab, the samples were emptied into sorting trays and the macroinvertebrates were extracted and sorted using tweezers and hand lenses. The various taxa were placed into corresponding labeled vials with isopropyl alcohol. Using a hand lens or microscope, the macroinvertebrates were identified to family and sorted into functional feeding groups. The number of individuals representing each functional group was analyzed with an ANOVA that included position (above or below), dam, and position * dam. Abundances were square-root transformed prior to analysis to improve normality. We also analyzed with the same model the proportion (also square-root transformed) of total individuals within each sample that fell into each functional group. Finally we analyzed the abundances and proportions of different functional groups with a three-way ANOVA that included position, dam, functional group, and all possible interactions. We tested for differences in temperature and dissolved oxygen above and below dams with paired t-tests. All analyses were done in JMP Pro 12.

RESULTS
The stream included five functional groups: collector/gatherers, predators, filter feeders, grazer/scrapers, and shredders (Table 1).

The dissolved oxygen content below dams was significantly greater than the dissolved oxygen content above dams ($t_{7}=2.40, p=0.05$, $df=7$; Figure 1). There was no difference in water temperatures above versus below dams ($t_{7}=0.45, p=0.67$, $df=7$; Table 2). Our observations confirmed our expectations that substrates were finer above dams than below.
Table 1. Total number of each taxa captured above and below dams.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Functional Feeding Group</th>
<th>Below</th>
<th>Above</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damselflies (Odonata)</td>
<td>Predator</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Dragonflies (Odonata)</td>
<td>Predator</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Non-segmented flatworms (Platyhelminthes)</td>
<td>Predator</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Caddisflies (Trichoptera: Hydropsychidae)</td>
<td>Filter Feeder</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>Mayflies (Ephemeroptera: Heptageniidae)</td>
<td>Scraper</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Beetles (Coleoptera: Ptilodactylidae)</td>
<td>Shredder</td>
<td>17</td>
<td>37</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>Collector-Gatherer</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Midge (Diptera: Chironomidae)</td>
<td>Collector-Gatherer</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>True bugs (Hemiptera)</td>
<td>Predator</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Black flies (Diptera: Simuliidae)</td>
<td>Filter Feeder</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>Aquatic worm (Oligochaete)</td>
<td>Collector-Gatherer</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Adult beetles (Coleoptera)</td>
<td>Collector-Gatherer</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

The proportions of functional groups were very similar above versus below dams (Figure 2), and there was little or no variation among dams (Table 3).

The numbers of individuals representing each functional group were also very similar below the dams (Table 4, Figure 3). There tended to be more filter feeders above the dams than below, although the difference was not quite significant ($F_{7,16}=3.62$, $p=0.08$). There was some variation among dams in the numbers of filter feeders ($F_{7,16}=4.61$, $p=0.01$) and shredders ($F_{7,16}=3.89$, $p=0.01$) found at different dams.

The results of a three way ANOVA showed that, overall, there were more macroinvertebrates above dams than below dams (Figure 4; $F_{1,80}=7.21$, $p=0.01$). The number of macroinvertebrates also varied among dams ($F_{7,80}=5.69$, $p=0.0001$), and there were clear differences in the numbers of macroinvertebrates among functional groups ($F_{7,80}=5.21$, $p=0.001$).
In spite of differences in total abundances, there were no differences in the proportions of functional groups above vs. below dams ($f_{7,65}=0.09, p=0.75$). Nor did we find significant differences in the proportions of functional groups among dams ($F_{7,65}=0.31, p=0.95$). Some functional groups were more abundant than others, but the relative proportions did not differ above vs below dams ($f_{7,65}=5.53, p=0.001$).

Table 2. Water quality measurements recorded at each dam (in order from upstream to downstream).

<table>
<thead>
<tr>
<th>Dam</th>
<th>DO (mg/L) Top</th>
<th>DO (mg/L) Bottom</th>
<th>Temperature (°C) Top</th>
<th>Temperature (°C) Bottom</th>
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</table>

DISCUSSION
We found no clear relationships between the community structure of macroinvertebrates, and therefore putative niches, associated with natural dams in our study stream. Although dams may not impact the proportion of functional feeding groups in a community, dams clearly affected the habitat for macroinvertebrates. That is, the proportional representation of each functional group did not change, even though total abundances of macroinvertebrates were higher above dams than below. Thus, the environment below dams is different enough from the environment above dams to have an effect on macroinvertebrate populations. This result also indicates that our sampling was probably sufficient to pick up changes in communities composition if it existed. Dams affect macroinvertebrate habitats, but apparently do not greatly affect the niches.

Our results are more consistent with the neutral hypothesis for species distribution than the niche theory. According to the niche theory, we would have found differences in structure above and below dams, assuming that functional groups occupy different niches in the system. Instead, we found that changes in environment did not significantly alter community composition. The distribution of macroinvertebrate taxa seems to be dictated by chance more than by competition for resources.

This is consistent with the life history of benthic macroinvertebrates. Of taxa that we sampled, there is much chance in where larvae occur; adult stoneflies, mayflies, dragonflies, and other macroinvertebrate taxa lay their eggs on the surface of the stream and the current carries their eggs away until they stick to a sub-surface feature. Further undirected dispersal occurs via larval drift. Without niche partitioning, this would produce an approximately random distribution consistent with that which was observed, suggesting that the neutral theory may explain species distribution in this stream.

Table 3. An ANOVA comparing the proportion of invertebrates in each functional group found above versus below each dam showed no difference between functional group proportions above versus below each dam. Error degrees of freedom = 16.

<table>
<thead>
<tr>
<th>Source</th>
<th>Collector/Gatherer</th>
<th>Predator</th>
<th>Filter Feeder</th>
<th>Grazer/Scraper</th>
<th>Shredder</th>
</tr>
</thead>
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<tr>
<td></td>
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<td>P</td>
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<tr>
<td>P x D</td>
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<td>0.38</td>
<td>0.90</td>
<td>1.23</td>
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</table>
Table 4. An ANOVA comparing the number of invertebrates found in each functional group above versus below each dam showed no difference between functional group counts above versus below each dam, although there were differences in the number of filter feeders and shredders found between the dams. Error degrees of freedom = 16.

<table>
<thead>
<tr>
<th>Source</th>
<th>Collector/Gatherer</th>
<th>Predator</th>
<th>Filter Feeder</th>
<th>Grazer/Scraper</th>
<th>Shredder</th>
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<td>0.86</td>
</tr>
</tbody>
</table>

Other explanations for species distribution in the context of this study are worth examination. For example, an alternate explanation is that variation in stream habitat is relevant to community structure at a smaller scale than we measured. Macroinvertebrates may not have the ability to maintain a structured community around features that are significantly larger than the substrate they live in. Stream habitats are patchy, and differences in substrate within a meter of the sample location may have a larger effect on community structure than the differences above and below a dam. If we had instead sampled a square meter plot at random points along the stream we may have found structured communities on this smaller scale.

In addition, it may be possible that strong top-down controls hid the variation in community structure above and below dams. We found a much larger proportion of predators in the system than would be expected for a temperate stream (see Figure 2-3). Thus, the number of individuals in the functional feeding groups may have been limited by predation more than resource abundance. Predation may reduce population size, which would reduce resource competition and limit structuring via different feeding niches, making structured niches unnecessary. If true, the patterns in functional feeding groups should become more evident were it possible to do predator removal experiments.

Our study provides an example in which neutral theory may better explain species distributions than niche theory. It is plausible that in situations in which resource competition is low, species will not depend on a narrow niche but rather will survive wherever they are dispersed. In environments in which species disperse in an approximately random manner, this will produce a random species distribution similar to the one observed in this tropical stream. However, it is important to acknowledge that our study was limited in scope and intensity. More research on dispersal biology and species assemblage in stream communities would further our understanding of the relationship between niche and neutral theory.

Understanding the processes that influence biodiversity is increasingly important as humans continue to reduce the extent of the ecosystems in which most of the planet’s diversity exists.

ACKNOWLEDGEMENTS:
We would like to thank Matt, Madi, and Braden for all their invaluable help and encouragement. Thanks also to Braden for thinking of our title. Thanks are due to the macroinvertebrates who died aiding us in enhancing our understanding of the natural world.
DENSITY-DEPENDENT EFFECTS ON NECTAR ROBBERY IN *IPOMOEA TRIFIDA*

LEIGH M. MOFFETT

Faculty Editor: Matthew Ayres

Abstract: Attracting pollinators is key to the reproduction of many angiosperm plants. Nectar robbers can pose a challenge to pollination success for some plants, especially those with long corollas. I tested whether nectar robbers of *Ipomoea trifida*, (the Stripe-tailed Hummingbirds), exert their strongest effects in high density patches of flowers. As predicted, the incidence of nectar robbery was highest in high density flower patches (as high as 50-70%). However, there was no relationship between nectar robbery and seed production. Optimal foraging theory provided a framework for understanding patterns and consequences of nectar robbery in *I. trifida*.

Key words: Eupherusa eximia, optimal foraging theory, pollination

INTRODUCTION
Species interactions can exert selection on plant reproductive strategies. For many species of plants, reproductive success depends on factors that attract potential pollinators such as the number of flowers on a plant, the spacing between flowers, the amount of nectar rewarded, and flower coloration. Species with long corollas sometimes offer greater nectar rewards to attract visitors that have become specialized for imbibing their nectar while pollinating the plant. However, some species of birds and insects, known as nectar robbers, have evolved ways of accessing nectar without pollination. One class of nectar robbery involves piercing the base of corollas to retrieve the nectar reward, without entering the flower and therefore without there being any chance of pollination (Lara & Ornelas 2001). Robbery can have strong effects on the fitness of individual plants by depleting nectar rewards and therefore visitation by potential pollinators (Maruyama et al. 2015).

Local plant density is one environmental feature that could influence the incidence of nectar robbery, and therefore affect the fitness of plants. If nectar robbers act as optimal foragers, one could expect that they would concentrate their foraging in relatively large and concentrated patches of flowers. If so, and if nectar robbery reduces pollination success, then nectar robbers could introduce density-dependence into the population dynamics of the plants on which they forage: i.e., per capita plant reproduction would tend to be high where local abundance is low, and low when local abundance is high. I tested this hypothesis with *Ipomoea trifida* (Convolvulaceae), a species of morning glory, which is an attractive target for nectar robbers because of its long narrow corolla and generous nectar rewards. I also tested the prediction that the incidence of nectar robbery would be highest and seed set lowest, in relatively large dense patches of *I. trifida*.

METHODS
From 18 to 19 January 2017, I collected data on eight different patches of *Ipomoea trifida* within a four kilometer radius of the Monteverde Biological Research Station in Monteverde, Costa Rica. The majority of patches (six) were within approximately 400-meters of the research station. In each patch, I counted the total number of open flowers, the number of flowers that had been robbed (by inspection of the lower corolla for punctures), and the number of seeds. I then measured the length and width of the patch using a tape measure to calculate patch area. These measurements were used to analyze relationships among robbery frequency, patch flower density, and pollination. For example, the number of seeds relative to the numbers of flowers within a patch provided a measure of pollination success. I also calculated flower density by dividing the number of flowers in the patch by the patch area. Flower densities were log transformed to improve normality prior to inclusion in a linear regression analysis.

To learn the identity of nectar robbers and potential pollinators I observed each patch for a minimum of ten minutes and recorded all flower visitors. I observed the largest patch, Patch 1, for several hours over the course of two days. I identified visitors to Patch 1 either by sight or by
Monteverde

capturing the insect using a butterfly net and bee
squeezers. To test for spatial heterogeneity in
nectar robberies and seed set, I measured the
height of ten random flowers in Patch 1 and tested
for differences in the average height of robbed and
un-robbed flowers.

RESULTS
There was variation in the proportion of robberies
among patches: high of 0.67 (Patch 8) and a low
of 0.04 (Patch 4). Patch 1 was the largest patch in
terms of flower density and appeared to have the
most visitors, mostly bees (Hymenoptera:
Andredidae) and butterflies (Lepidoptera:
Hesperiidae). During an observation of Patch 1, I
observed the nectar robber, *Euperusa eximia*
(Stripe-tailed Hummingbird), piercing holes in the
corollas of multiple flowers within the patch. I
also observed a Variegated Squirrel (*Sciurus
variegatoides*) consuming entire flowers.

The density of flowers within a patch was
positively correlated with an increase in the
frequency of robberies (slope=0.13±0.07, *p*=0.09,
*r*=0.40; Figure 1) but was unrelated to the ratio of
seeds to flowers (a proxy for pollination success)
(slope=0.02±0.04, *p*=0.64, *r*²=0.04; Figure 2).
Therefore, there was no evidence that nectar
robbery influenced pollination success. In Patch 1,
flowers that had been robbed tended to be higher
off the ground than un-robbed flowers: 1.29 ± 0.18
m versus 0.47 ± 0.18 m (*t*_8=3.14, *p*=0.01).

Figures 1 and 2.

DISCUSSION
There were some relations among flower density,
robbery incidences, and pollination events. A
higher density of flowers in a patch leads to an
increase in the number of robberies in that patch,
assuming that the robber, *E. eximia* (Stiles &
Skutch 1989), acts as an optimal forager and
reaches the patch prior to pollinators (Figure 3a).
This would mean that pollinators would spend less
time in the patch (assuming they also forage
optimally) due to a lower density of flowers with
(Figure 3b). Furthermore, it is plausible that these
pollinators will need to visit more patches to
account for the depressed energy gain from a
robbed patch as seen on the optimal foraging
curve. If a potential pollinator visits more patches,
it is likely facilitating pollination more evenly
across patches regardless of the patch flower
density. The discovery that *E. eximia* was the
nectar robber allows for this line of logic.

However, the time it takes to travel from patch
to patch may alter the predictions from this theory.
For instance, it is presumable a greater investment
for small pollinators (typically insects such as bees
and butterflies) to travel to a patch effectively
farther away than it is for the large robber (*E.
eximia*) (Figure 3). Thus, it is plausible that the
increase in travel time for an insect pollinator
(versus a hummingbird robber) will increase the
amount of time a pollinator spends in a robbed
patch according to the optimal foraging theory.
This would increase the number of flowers a
pollinator has to visit in one patch to make up for
the increase in travel time. This would facilitate the pollination of that patch by increasing the possibility of gamete exchange with other plants, therefore increasing the number of seeds not in just that patch, but also in other patches. Therefore, the prediction that denser patches would experience more robbery and more pollination events was incorrect.

The weak positive correlation between incidence of robbery and seeds relative to flowers may be a coincidence. There is no plausible biological mechanism that would lead the plant to increase the density of flowers within a patch to increase the number of robberies. This often appears to be an antagonistic interaction (Maruyama et al. 2015) and it is unlikely that the plant optimizes floral density to cause more uniform pollination. Therefore, it is unclear whether *I. trifida* takes advantage of factors such as flower density within a patch to indirectly alter its reproductive success. There must also be other factors that affect robbery beyond flower density within a patch. For example, variation in the height of flowers apparently also affects robbery incidences, perhaps because it is more dangerous or challenging for a hummingbird to forage (by robbing flowers) near the ground. Further research with more patches that have height variation between flowers would be necessary to further test this result. Furthermore, other factors such as location, elevation, weather, and differences in surrounding vegetation may alter the likelihood of robbery. It would be informative if further research were conducted in a larger study area. A deeper understanding of interspecific behavioral interactions and how these influence plant reproductive strategies is increasingly relevant in a rapidly changing world, not just in nature but also in food production systems that depend on animal pollination.

ACKNOWLEDGEMENTS
Thank you to Dr. Matthew Ayres and the teaching assistants for all of their support and help with the many components of this project. Thank you to the students of the 2017 Biology FSP for their encouragement during this study.

AUTHOR CONTRIBUTIONS
The author made all contributions.

LITERATURE CITED
DENSITY AND PARASITISM OF BOMERIA PLANTS

SAMUEL R. GOCHMAN AND LOUISE J. BARIAS

Faculty Editor: Hannah ter Hofstede

Abstract: Plants have evolved many strategies to combat predation and improve their fitness. Aggregation might increase the risk of predation due to greater visibility or may dilute the risk for each plant through predator swamping. Bomeria inflorescences are found in aggregated groups, potentially employing the latter strategy against Diptera that parasitize the inflorescences and damage their reproductive organs. To test the effect of aggregation on the prevalence of parasitism, we collected 33 inflorescences of Bomeria with a total of 809 flowers at the Cuerici Biological Station in Costa Rica. At a fine scale, the number of flowers within an inflorescence did not affect the proportion of parasitic damage to the inflorescence. Additionally, comparisons of damage due to parasitism between aggregated and isolated inflorescences indicated that high density of inflorescences does not reduce the incidence of parasitism. At a larger scale, geographical locations of the inflorescences seemed to affect the rate of parasitism to the inflorescences; however, the variance of parasitic damage between aggregated groups of inflorescences was minimal. Instead, parasitic damage to inflorescences varied substantially, indicating that there was no effect of the number of flowers per inflorescence, the number of inflorescences in an aggregation or the geographic location of the inflorescences on parasitic rate. Thus, aggregation may not be an effective defense strategy for all plants.

Key words: aggregation, Bomeria, Diptera, inflorescence, parasitism

INTRODUCTION
Predators depend on the presence of prey items, and so predation is often controlled by the density of prey. To combat predation such as herbivory and parasitism and increase their reproductive fitness, plants have evolved a diverse range of strategies including spatial organization. When plants grow in aggregated groups, an individual plant may be less likely to be targeted by parasites, diluting the probability of predation across all plants and increasing the fitness of individual plants through predator swamping. This strategy would ultimately result in high-density plant growth. Alternatively, plants that aggregate may be more conspicuous, drawing more predators and reducing the reproductive fitness of each plant. In this case, plants would ultimately grow in relative isolation.

A plant that may employ aggregation as a defense strategy is Bomeria, a genus of herbaceous vine endemic to Costa Rica that can be found at high altitudes (Gargiullo et al. 2008). At Cuerici Biological Station, a Bomeria plant contains one inflorescence composed of red-orange flowers. Plants can grow in isolation, resulting in an isolated inflorescence, or they can grow close together, resulting in aggregated inflorescences with each inflorescence arising from a different plant. Parasitic flies can lay their eggs inside a flower. After hatching, the larvae consume the reproductive organs of Bomeria flowers until they pupate and then emerge as adults. Thus, damage to the anthers in a flower can reduce the fitness of the plant.

Aggregation may influence the overall reproductive fitness of the Bomeria inflorescences by influencing the incidence of parasitism. Aggregation may affect parasitism at multiple scales, including the density of flowers within inflorescences, the density of aggregations of inflorescences, and the geographical layout of aggregations of inflorescences (Fig. 1). At a fine scale, the number of flowers within an inflorescence may affect the proportional parasitic damage to that inflorescence. If a high number of flowers in an inflorescence swamps parasites, an inflorescence with many flowers should have relatively low damage due to parasites. However, if an inflorescence with many flowers is especially conspicuous to a parasite, then an inflorescence with a high number of flowers should have relatively high damage.

At an intermediate scale, the density of inflorescences may impact the damage done to any inflorescence. If aggregated inflorescences swamp parasites, the incidence of parasitic dipteran larvae is expected to be lower in the flowers of aggregated inflorescences than in flowers of isolated inflorescences. However, aggregation may make the inflorescences more conspicuous,
attracting dipterans and increasing the incidence of parasites.

Alternatively, aggregation may not be a defense against predation for inflorescences, thereby producing no observed difference in the incidence of parasitism for aggregated or isolated inflorescences. At a coarse scale, properties of the locations in which inflorescences are found may have an effect on parasitism. There may be areas in which there is more or less damage inflicted on inflorescences due to properties of the local environment.

**METHODS**

We collected *Bomeria* inflorescences at the Cuerici Biological Field Station in Costa Rica. Two or more inflorescences within a five-meter radius were classified as an “aggregate” and inflorescences found at least five meters away from the nearest inflorescence were classified as “isolated inflorescences.” For each collected inflorescence, we counted, separated, and dissected the flowers and extracted and counted the dipteran larvae or pupae found inside the flowers. We also noted the presence of other taxa in the flowers. We analyzed the effect of multiple scales (Fig. 1) on the parasitic damage to *Bomeria*.

**Flowers within an inflorescence**

To understand how parasitism and damage were related, we calculated the overall proportions of flowers that were damaged (contained parasites or evidence of parasites) and contained parasites without damage. The reproductive organs were damaged when they were buried into by larvae, had holes, or were soft and brown. To determine if the number of flowers within an inflorescence affected the parasitism of *Bomeria* at the fine scale, we compared the abundances of flowers contained in each inflorescence to the proportional parasitic damage to the inflorescence using a linear regression in JMP Pro V. 13.

**Inflorescences and density**

To test for the effect of the density of inflorescences on the proportion of damaged flowers in an inflorescence, we recorded the number of inflorescences in each aggregate (i.e., aggregate density) and performed a one-way ANOVA between aggregated inflorescences and isolated inflorescences. Since aggregate density is a continuous variable, we tested the relationship between aggregate density and the proportion of damaged flowers in each inflorescence using a linear regression.

**Geographical location of inflorescences**

To observe potential large-scale differences in parasitism among different areas of the site, we investigated the potential effect of geographical location of the aggregates of inflorescences by recording the longitude and latitude for each aggregate and isolated inflorescence. We also considered that the geographical placement of aggregates or isolated inflorescences may have no effect on the proportion of damaged flowers in an inflorescence. To see if the proportion of damaged flowers was simply different for each aggregate or isolated inflorescence, we used a hierarchical analysis of variance to estimate the variance in the proportion of damaged flowers between the sampled aggregates (and isolated inflorescences) and between their residuals.
RESULTS
We collected 33 *Bomeria* inflorescences with a total of 809 flowers, each inflorescence containing an average of 24.5 ± 8.9 flowers. The inflorescences were found in 19 aggregates or isolated areas. The distribution of the number of inflorescences per aggregate was not constant (Fig. 2).

We collected a total of 404 dipteran larvae from the all parasitized flowers. In the inflorescences, we found other invertebrates such as spiders (order Araneae), caterpillars (order Lepidoptera), a neuropteran larva, mites (order Acari), beetles (order Coleoptera) and a cricket (order Orthoptera). When we found mites in an inflorescence, we found no dipteran larvae and vice versa. We also found a translucent invertebrate egg in one flower surrounded by frass. In the damaged flowers containing dipteran larvae, we most often found those parasites burrowed into the anther, where they consumed the internal organic matter. Some of the parasites were found between a petal and sepal, and the pupae (n = 28) were found in the same place but closer towards the base of the flower.

Flowers within an inflorescence
Forty-two percent of all flowers surveyed were damaged and contained parasites or showed evidence of parasitism as indicated by decay of the anthers and sometimes presence of frass. Flower damage was used as an indication of parasitism and 47% of damaged flowers contained parasites at the time of sampling. In some instances, more than one parasite was found in a single anther or several smaller parasites were clustered under a petal. When at least one parasite was present in a flower, there was usually damage to the flower. Less than 1% of the flowers contained parasites but had no damage (Table 1). The number of flowers within an inflorescence did not significantly affect the proportion of damaged flowers in an inflorescence ($F_{1,31} = 0.38$, $P = 0.54$).

<table>
<thead>
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<th>Flowers with Parasites</th>
<th>Flowers with No Parasites</th>
<th>Total flowers</th>
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<tbody>
<tr>
<td>Intact</td>
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<td>471</td>
</tr>
<tr>
<td>Damaged</td>
<td>156</td>
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</table>

Table 1. Distribution of flowers that were intact or damaged and contained parasites or no

Inflorescences and density
At an intermediate scale, 55% of all inflorescences had 40 to 100% of their flowers damaged. The proportion of damaged flowers per inflorescence was not significantly different between aggregated and isolated inflorescences (Fig. 3, $F_{1,17} = 0.43$, $P = 0.52$). There was also no effect of the density of inflorescences on the proportion of damaged flowers per inflorescence ($F_{1,31} = 0.004$, $P = 0.95$).

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Geographical location of inflorescences
At a larger spatial scale, the geographical locations of aggregates of inflorescences and isolated inflorescences seemed to affect the incidence of parasitism. The southwest corner of our study site contained areas in which the proportion of damaged flowers was relatively low (Fig. 1). However, less than 0.2% of the variance in the proportion of damaged flowers was between aggregates of inflorescences and isolated inflorescences. Conversely, over 99.8% of the variance in the proportion of damaged flowers was found between inflorescences.

DISCUSSION
Our data provide insight into the interaction between Bomeria flowers and their dipteran parasites at multiple spatial scales. Almost half of all collected flowers were damaged by parasites, greatly reducing the reproductive potential of those plants. Of these flowers that showed evidence of parasitism, over half contained no parasites at the time of collection. Often, these flowers were fully open suggesting that they were at a relatively late stage of development. Further, our analysis of proportional damage as a function of the flowers’ developmental state (closed/open) suggested that damage did not vary with age. It follows that dipteran larvae were once present but emerged as adult flies before our collection, and that age of the flowers at collection does not reflect the flower choice of the fly. Regardless, it is evident that the dipteran larvae develop by exploiting the plant’s reproductive parts, reducing the plant’s fitness in the process.

To see if the number of flowers within inflorescences affected the incidence of parasitism (and reproductive fitness) of inflorescences, we evaluated the effect of flower density on the damage to inflorescences. The number of flowers within an inflorescence had no effect on the incidence of parasites, suggesting that flies may oviposit in an inflorescence regardless of the number of flowers available in that inflorescence. Further, we collected several inflorescences in which the number of larvae per flower in an inflorescence was either very large or very small. This observation may indicate that dipteran flies randomly select plants for oviposition. It is also possible that dipterans’ fecundity is regulated by their size rather than the properties of the inflorescence. In addition, multiple flies may lay eggs in the same inflorescence. This may dilute the effect on the incidence of parasitism by the inflorescence’s flower density or proximity to other inflorescences.

At an intermediate spatial scale, the densities of inflorescences did not affect the incidence of parasitism when viewed as either a categorical (aggregated or isolated) or continuous variable. Therefore, the number of inflorescences in an aggregate (and thus closeness of inflorescences) was not a key factor in the incidence of parasitism. Instead, the incidence of parasitism for each inflorescence seemed to be independent of the number of surrounding inflorescences.

We next looked to a larger spatial scale for geographical patterns in the data. Initially, a map of our study site visually indicated an area with lower rates of parasitism. This pattern could have been explained by properties of the local environment that are less conducive to the survival of larvae such as variations in humidity, temperature, and exposure. However, the variance of proportional damage to inflorescences between aggregates was extremely low, suggesting that rates of parasitism were fairly consistent among aggregates of inflorescence.

Variance in proportional flower damage revealed that individual inflorescences had very different rates of parasitism, regardless of their aggregation with each other. This result suggests that the number of flowers, density of inflorescences, and geographical layout of aggregates had little effect on flower damage and instead, inflorescences may be characterized as distinct entities. In many cases, a parasite-free inflorescence neighbored a heavily infested one, showing that each inflorescence may be prone to its own number of fly larvae. These results point to the fly’s behavior strongly controlling the parasitic interaction in which an adult fly invests most of its eggs into one inflorescence. Future studies could make comparisons between plant-parasite systems to explore this potential low-energy dispersal strategy.

One predicted strategy for improving Bomeria fitness included reducing conspicuousness via a low abundance of inflorescences within an aggregate, and another included diluting the chances of parasitism for each inflorescence via swarming with a high abundance of...
inflorescences. However, we observed neither phenomenon in Bomeria plants. Our data show that the density of inflorescences did not affect parasite abundance in the Bomeria plant system and that future studies should consider investigating other characteristics of individual plants to determine why some flowers and inflorescences are parasitized and others are not.

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AUTHOR CONTRIBUTIONS
All authors contributed equally to this project.

LITERATURE CITED
CHEEPER BY THE DOZEN: THE PURPOSE OF REGULAR *COLIBRI THALASSINUS* VOCALIZATIONS

FRANCESCA C. GOVERNALI, CAROLINE V. KOLLER, LEIGH M. MOFFETT, AND MARIKO C. WHITENACK

Faculty Editor: Hannah ter Hofstede

Abstract: In organisms across the animal kingdom, males that command a territory are thought to be more dominant and have a higher likelihood of attracting females. Though mate attraction and territory defense are connected, successful mate attraction is ultimately what gives an individual higher fitness. Vocalizations can be used to communicate both territoriality and courtship. In each case, exerting energy to vocalize is necessary. However, whether this energy is more likely to be used to attract mates or defend territory is unclear. We spent two days studying *Colibri thalassinus* in the forest and trails around Cuericí Biological Research Station in Costa Rica. We made auditory observations of 84 individuals to record vocalization rate and the presence or absence of nearby conspecifics. We analyzed vocalization rate before and after a playback of a conspecific male call. Our findings demonstrated that metabolic drivers, such as time of day and temperature, did not influence vocalization rate. This suggests that these vocalizations are not intended for territorial defense. Instead of being impacted by outside factors, the vocalization rate of male *C. thalassinus* could be a fixed action pattern, likely optimized to attract females.

Key words: *Colibri thalassinus*, fixed action pattern, hummingbird, playback

INTRODUCTION

Individual fitness is directly linked to reproductive success and the tradeoffs it involves. If organisms devote too much time to defending territory, they may lose out on attracting mates. At the same time, having a desirable territory could make them more attractive to potential mates. If an individual spends too much time or energy on territorial displays and not foraging, it may threaten its own survival and thus its reproductive success. Ultimately, an individual’s fitness is determined by whether or not it is able to reproduce. To attract mates, individuals often invest in vocalizations or displays. However, organisms such as hummingbirds use vocalization for both defending territories and attracting mates. For territorial hummingbirds, these kinds of tradeoffs are especially important for maintaining territory but also ensuring that they have enough energy and resources to find mates and reproduce.

Some species of hummingbirds guard territories primarily for foraging purposes. Territorial species near Cuericí Biological Station in Costa Rica include the Fiery-throated hummingbird, Volcano hummingbird, and Green Violet-ear hummingbird. Male hummingbirds often maintain these territories by vocalizing their ownership of a specific area to their conspecifics (Stiles & Skutch 1989). *Trochilidae* spp. spend more time foraging when there is no other vocalizing conspecific nearby, demonstrating that hummingbirds will change their behavior in response to territorial calls (Carter et al. 2012). On the other hand, males often vocalize or perform courtship displays for female hummingbirds. Therefore, it is reasonable to assume that a territorial species like the Green Violet-ear (*Colibri thalassinus*) will alter its vocalizations depending on the identity of the receiver. In order to maintain a territory, it is likely that call frequency will increase in the presence of another vocalizing conspecific if the intended receivers of the calls are other males. However, vocalization may not change at all if the intended receivers are female hummingbirds.

*Colibri thalassinus*, and hummingbirds in general, may have a fixed vocalization rate for calls intended to attract mates. Males may have evolved sensory exploitation tactics to vocalize at a rate that takes advantage of a call frequency that is most attractive to females. However, if the vocalizations are instead used primarily for territorial calls, the rate may change based on the presence of competitors or environmental factors. This could be due to tradeoffs made by males in balancing territorial interactions with foraging.
behavior. As homeotherms, *C. thalassinus* maintain a body temperature of approximately 37°C. When the difference between their body temperature and ambient air temperature grows, their metabolic rate increases to stay warm. With a higher metabolism at colder temperatures, hummingbirds may have less available energy to vocalize, and may have to make a tradeoff between metabolism and vocalizing for territorial interactions. Higher temperatures may allow *C. thalassinus* to reduce energy spent on keeping warm and, therefore, energy is freed up for defensive vocalizations. Time is also a factor in territorial vocalizations, because *C. thalassinus* cannot forage at night, and therefore must forage in the early morning rather than defending their territories. Alternatively, the territorial Fiery-throated hummingbird (*Panterpe insignis*) has been found to spend more time vocalizing in the morning due to increased nectar production and more abundant interspecific competitors during that time of day (Taliaferro and Little 1996). Despite these findings, it would seem that as temperature rises over the course of the day and the metabolic rate of hummingbirds decreases, they may have more available energy to vocalize. Given this, if vocalizations are primarily for territorial defense, then *C. thalassinus* would be likely to vocalize more during the warmest parts of the day.

**METHODS**

We conducted research from 25 to 26 January 2017 along a trail, a road, and four open areas around the Cuericí Biological Research Station in Cartago Province, Costa Rica. The primary sampling periods were from 0800 to 1100 and 1300 to 1500. When we identified a *C. thalassinus* vocalization, we recorded the time and remained stationary for the duration of the observation period. Two listeners counted vocalizations for thirty seconds. We then played a pre-recorded 30-second *C. thalassinus* vocalization (see supplemental material). The rate of the recorded call was 132 vocalizations per minute. We conducted a final listening period after the playback, counting the number of vocalizations in 30 seconds. We averaged the vocalizations simultaneously recorded by each observer. We also noted whether or not there was another *C. thalassinus* individual vocalizing nearby. We did not record observations when an individual call was indistinguishable from other calls, due to the potential for miscounting.

We only recorded observations of *C. thalassinus* individuals. Four other species (Magnificent, Volcano, Fiery-throated and Gray-tailed Mountain-gem hummingbirds) are common in this area, but vocalizations are distinguishable from that of *C. thalassinus*. One individual may have been observed multiple times during the study, but not twice in a row as we ensured that we moved up the trail, out of earshot of the previous individual, before recording a different individual. We attempted to identify the vocalizing bird using Bushnell binoculars (8x42), but were not always able to locate the individual through the foliage. To supplement our auditory observations, we spent one 20-minute period noting behavior of four hummingbirds of different species (*C. thalassinus* and *Selasphorus flammula*) at one flower patch behind the classroom. During this period we also observed reactions of heterospecifics to the recorded *C. thalassinus* playback. Temperature was recorded using a HOBO temperature logger placed in the forest and recorded temperatures were matched to time of observation.

We used a paired t-test to determine the difference in vocalizations per minute before and after the playback. We compared mean vocalization rate for individuals with and without conspecifics present, and before and after playback with a t-test. We performed a two-way ANOVA to test the interaction of playback and presence of another vocalizing individual in relation to vocalization rate. We conducted a linear regression of temperature against vocalizations per minute, and conducted a second linear regression for time of day versus vocalization rate. All statistical analyses were conducted in JMP Pro 13 and Microsoft Excel.

**RESULTS**

Of the 84 *C. thalassinus* individuals we observed, 53 were within hearing distance of another individual. The overall mean number of vocalizations per minute was 119.80.
Vocalizations per minute did not differ before and after playback ($t = 1.16$, $P = 0.25$, df = 76; Fig. 1), and there was no interaction between playback and presence of another vocalizing individual ($F_{1,150} = 1.42$, $P = 0.23$). Additionally, vocalization rate was unrelated to the presence of other vocalizing individuals ($t = 1.68$, $P = 0.10$, df = 44; Fig. 2). Ambient temperature had no impact on average vocalizations per minute ($F_{1,75} = 0.60$, $P = 0.44$; Fig. 3). Similarly, time of day did not impact vocalizations per minute ($F_{1,75} = 0.42$, $P = 0.52$; Fig. 4).

In our 20-minute observation period, multiple Volcano hummingbirds ($S. flammula$) were seen foraging and loafing. We did not observe a change in their behavior upon playback of the $C. thalassinus$ vocalization. A visual observation during data collection allowed us to make a positive identification of a $C. thalassinus$ during vocalization. In this case as well, behavior of the individual and vocalization rate did not change after the playback.
DISCUSSION

There was no difference in vocalization rate of male *C. thalassinus* in the presence or absence of other vocalizing hummingbirds and no response to the playback of male *C. thalassinus* calls. This suggests that vocalization rate is a fixed action pattern and that these vocalizations are targeted at female hummingbirds. Additionally, *S. flammula* did not alter their behavior during or after the playback. Both *C. thalassinus* and *S. flammula* are known to have conspecific and heterospecific territorial interactions (Stiles & Skutch 1989), so the lack of reaction we observed also suggests that male *C. thalassinus* use these vocalizations to advertise themselves to females. *Colibri thalassinus* females are known to build nests in their own reproductive territory (Stiles and Skutch 1989). This behavior could devalue the importance of male territory and lead to males investing less time in male-male territorial displays and more time in attempting to attract females.

Ambient temperature and time of day had no effect on *C. thalassinus* call rates. This suggests that the vocalizations are not male-male territorial calls, as individuals would likely change call rate in response to ambient factors influencing metabolism. The lack of change could be because the calls are part of a fixed action pattern intended to communicate male availability to females. Male *C. thalassinus* could have converged on a vocalization rate of 120 vocalizations per minute as a sensory exploitation of a female bias towards that rate.

Future research on hummingbird vocalizations could include observing focal hummingbirds around sunrise to calculate time budgets for behaviors including vocalizations, foraging, loafing, and preening. Observations of female hummingbirds’ behavior before and after a playback could also provide insights into the purpose of male hummingbird vocalizations. Male vocalization convergence could be explored in further studies through phylogeny and experimental tests on vocalization rates of closely related species.

Male *C. thalassinus* still face tradeoffs between the energetic cost of calling to defend territory and reproductive success gained from attracting females. Because females may not value male territories as much as in other territorial species, it is beneficial for males to use regular calls to attract mates rather than to constantly defend a territory. Male *C. thalassinus* have devoted energy to vocalization for females and therefore, have converged on an optimized call rate because it increases individual fitness. They then invest energy in vocalizing to potential mates rather than to defend territory. As in species across the animal kingdom, ultimately, when energy is limited, the behavior that is prioritized is that which is most likely to lead to successful reproduction.

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LITERATURE CITED


IT'S GETTING HOT IN HERE: NONATIVE PLANTS OFFER THERMAL REWARDS TO NATIVE BEETLES

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Faculty Editor: Hannah ter Hofstede

Abstract: Introducing nonnative plants into a landscape can have dramatic results on the biological community. We examined whether thermal rewards play a role in the relationship between three nonnative plants in Cuerici, Costa Rica and a native beetle (family: Chrysomelidae). The three plants were daisies, lilies, and dahlias, all of which have flowers with a parabolic shape that is distinct from the pollination syndrome of downward- or upward-facing tubular flowers in native plants. The parabolic shape of the flowers suggests that the introduced plants might offer thermal rewards to insects. A relationship between the native ectotherms and the nonnative plants be mutually beneficial; the beetles be exploiting the natural heat-magnifying structures of the parabolic flowers, and increasing the rate of pollination for the flowers. To answer this question, we measured the thermal rewards of each type of flower, investigated the physiological benefit of heat to the beetles, examined whether beetles preferentially seek out thermal rewards, and studied whether beetles offer pollination rewards to flowers. Our results suggest that Chrysomelidae beetles engage in a mutually beneficial relationship with dahlias, daisies, and lilies. The beetles seek out thermal rewards, which lead to increased mating rates. Thermal rewards also benefit the plant by increasing pollination rates. Further research could examine the potential for other nonnative plants to increase the geographic range of herbivorous ectotherms through thermal rewards.

Key words: Chrysomelidae, ectotherms, native beetles, nonnative plants, thermal rewards

INTRODUCTION

When a nonnative plant species is introduced to a landscape, it might become invasive due to release from natural predators, be outcompeted by the native species that have adapted defenses, or find its own niche in the biological community. Nonnative plants might find a niche because they have a trait that has not previously been by native species. For example, certain plants have structures that magnify radiant energy and offer thermal rewards to native pollinators in colder climates. A study of darkly-colored eastern Mediterranean irises showed that honeybees spend more time on east-facing flowers, which can absorb radiant more radiant energy from the rising sun (Sapir et al 2006). This added heat to insects, especially ectotherms like the beetles at Cuerici, would boost metabolic rates, allowing insects to increase foraging or reproductive rates. This would also benefit the flowers, as the thermal rewards further incentivize pollinators to spend time at the reproductive organs of flowers.

According to the thermal reward theory, this heat benefit causes the pollinators to seek out the plants, thereby increasing pollination, benefiting both plant and pollinator.

Due to high year-round temperatures, most of Costa Rica seems an unlikely place for nonnative plants that offer thermal rewards to thrive. However, at Cuerici Biological Field Station, a high-elevation (2,500m) site, temperature becomes much more limiting to ectotherms. Dahlias, daisies, and lilies, plants from temperate zones, are examples of plants that might offer thermal rewards in an area mostly vacant of this adaptation. These nonnative plants are visually different than the native flora, with large inflorescences in a parabolic centralized structure, as compared to the local pollination syndrome of downward- or upward-facing tubular flowers. These parabolic shapes seem more likely to offer thermal rewards to pollinators plagued by cold temperatures than most native plants in Costa Rica. One such benefitting pollinator might be the native leaf-mining leaf beetle (family Chrysomelidae, subfamily Hispinae) we observed visiting these three types of nonnative flowers. Adults in this family feed on pollen from both native and nonnative plants (Borror et al 1992). The question is whether thermal rewards also play a role in the relationship between the flower and beetle species. The relationship might be mutually beneficial; the beetles might be
exploiting the natural heat-magnifying structures of the nonnative plants, or thermal rewards might have no role in structuring the relationship between flowers and beetles. The proprietor of Cuerici Biological Field Station has noticed an increase in the beetle population since he planted the flowers, and has observed the beetles travelling from flower to flower throughout the day, aiding in pollination. Thus, it seems the relationships between parabolic flowers and beetles is mutually beneficial. If this is the case, there must be supporting evidence for four postulates. (1) Dahlias, daisies, and lilies (the nonnative plants) capture heat. This would result in higher temperatures at the center of flowers than the surrounding environment. (2) Beetles benefit from thermal rewards. This would mean that thermal rewards result in increased rates of beetle survival and reproduction in the presence of thermal rewards. (3) Beetles seek out thermal rewards from flowers. This would result in beetles preferentially seeking out warmer flowers. (4) Plants with thermal rewards benefit from increased beetle visitation rates. In this case, plants would benefit from increased pollination rates.

**METHODS**

*Heat capture by flowers*

To measure the thermal rewards of three flower species (dahlias, daisies, and lilies), nine thermocouples recorded temperature information over a twelve-hour period on both January 25 and 26, 2017. All thermocouples were attached to a 1 cm³ black body object made of foam. For each plant species, a dense patch of the given flowers was identified. One thermocouple was placed in an area of constant shade and another was placed in an area of constant sun within each flower patch. For each plant species, a third thermocouple was placed in the center of a sun-exposed flower chosen at random. The thermocouples recorded temperatures every second from 0900 to 1700. For each thermocouple, the data were averaged across
every fifteen minutes (except for the thermocouple in the shade treatment of the lilies, which was already recording in 15 minute increments). Two of the thermocouples failed, so we were only able to compare all three temperature treatments in lilies (sun, shade, flower). We ran a General Linear Model in JMP Pro 13 comparing the average temperatures of each treatment throughout the day in the lily patch, as well as the available all-day temperature data for the two other species. We also ran a General Linear Model comparing the all-day temperature data of the daisy shade and flower treatments, and the dahlia sun and flower treatments. began.

Next, we repeated the same procedure at a higher temperature. We placed the enclosure into an open plastic bag and then placed the bottom, water-proofed side of the plastic bag into a warm water bath. We put a thermometer in the enclosure to measure air temperature. We waited until the thermometer read 25.5°C and then added the beetles. Finally, we repeated this procedure with a cool water bath so that the internal temperature of the enclosure was 18.5°C.

In the field, we collected data on beetle copulations on flowers during 20-minute observation periods on the morning of January 25. We counted the number of copulations on each flower and measured the temperature of the center of the flower and on the petals at the beginning and end of each observation period. We used a General Linear Model to examine the relationships between the percentage of beetles copulating for all the beetles on the flower and temperature and light condition. Dahlias were excluded from the analysis of copulations because we never observed more than one beetle on any single flower.

**Beetle heat preference** began.

To examine whether beetles preferentially seek out thermal rewards, five flowers of each species were experimentally shaded on January 26, 2017. The shading structures that covered the sun-exposed side of five of each flower species were constructed the night of January 25, 2017 and blocked the flowers from sunlight for all of the next day. The shades did not touch the plants and allowed for free airflow to minimize any accidental physical barrier preventing the beetles from reaching the shaded flowers. Each shaded patch was next to at least five un-shaded flowers of the same species.

We recorded hourly temperatures of one shaded flower and one adjacent sunny flower eight times between 0910 and 1610 on January 26, 2017. The temperature was taken at the center and petals of each flower. We also measured beetle abundance on the five shaded flowers as well as the five adjacent sun-exposed flowers for all three flower species. We examined the effects of temperature, shade, and flower species on beetle

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**Beetle physiological benefits**

We had previously noticed the beetles mating on the nonnative flowers, and, to test the relationship between number of copulations and temperature, we collected 21 male and 21 female beetles from the gardens at Cuerici Biological Field Station on January 25, 2017. We placed 7 females and then 7 males into an enclosure (Pop-up Port-a-Bug) at room temperature (21.5°C) and inserted a thermocouple (model Bat 12 thermometer) attached to a black body object to record temperature. We then counted the number of copulations between individuals over a ten-minute period.

We also ran a General Linear Model comparing the all-day temperature data of the daisy shade and flower treatments, and the dahlia sun and flower treatments.
abundance using a three-way ANOVA in JMP Pro 13.

We also collected data on flower temperature and beetle abundance on flowers during 20-minute observation periods in situ on the morning of January 25, 2017. We counted beetle abundance and location on each flower and measured the temperature of the center and petals at the beginning and end of each observation period. We used an ANOVA to examine the relationship between the temperature differential between centers and petals for each species and light condition (clouds or sun).

**Pollination benefits to flowers**

To examine whether beetles participate in pollination (and therefore benefit the flower), we counted the number of beetles visiting and leaving 7-10 flowers of each species over a twenty-minute interval. This process was repeated every hour between 0910 and 1610 on January 26, 2017. The temperature of the center and petal of each flower species was taken during each observation interval. The flower chosen from each species was exposed to the sun. We examined the relationship between flower temperature and beetle transitions for each flower species using a two-way ANOVA in JMP Pro 13.

**RESULTS**

*Heat capture by flowers*

The lily centers were, on average, hotter (19.5 °C) than both ambient sun (16.5 °C) and shade (15.6 °C) temperatures (F_{2,64} = 47.17, P < 0.001; Fig. 1). Mean daisy temperatures were significantly different from mean shade temperatures (F_{1,32} = 9.55, P = 0.004), but mean dahlia temperatures were not significantly different from mean sun temperatures (F_{1,32} = 0.72, P = 0.41). We were unable to take data for the remaining treatments as our thermocouples failed. All temperature readings varied significantly with time over the course of the day (lily: F_{32,64} = 4.45, P < 0.0001; daisy: F_{32,64} = 4.22, P < 0.001; dahlia: F_{32,64} = 78.44, P < 0.001).

*Beetle physiological benefits*

For the temperature controlled experiments, the number of copulations that occurred within ten-minute observation periods varied with temperature; there were 4 copulations at 18.5°C, 19 at 21.5°C, and 35 at 25.5°C.

When observing beetle behavior in the field, the percentage of beetles mating and flower temperature were positively related (F_{1,32} = 4.85, P = 0.035). The percentage of beetles mating and light level (sunny or cloudy), however, were unrelated (F_{1,29} = 3.33, P = 0.078). Lilies and daisies did not have significantly different percentages of beetles mating (F_{1,32} = 0.023, P = 0.88).

**Beetle heat preference**

The densities of beetles in experimentally shaded and un-shaded flowers were not significantly different (F_{1,42} = 0.76, P = 0.39). However, beetle density and temperature were positively related (F_{1,42} = 11.61, P = 0.002), and lilies had a higher overall beetle density (5.81±0.69) than daisies (0.44±0.69) or dahlias (0.31±0.68, F_{2,42} = 23.96, P < 0.001; Fig. 2).

Average temperatures were higher in daisies (21.52±0.66°C) than in lilies (18.80±0.66°C) or dahlias (18.38±0.67°C) (F_{2,91} = 6.63, P = 0.021). Beetle abundance was, however, higher on lilies (1.59±0.07 beetles/flower) than daisies (0.33±0.06 beetles/flower) or dahlias (0.02±0.06 beetles/flower) (F_{2,91} = 174.04, P < 0.001).
Within flowers, the center and petals did not have significantly different temperatures over the course of a day ($F_{1,91} = 2.18, P = 0.14$). However, when we included light condition in the model, the temperature difference between the center and petals was significantly higher during sunny observations ($4.14\pm0.23{\degree}C$) than cloudy ones ($1.36\pm0.13{\degree}C; F_1 = 97.67, P<0.001$). Beetle abundance was greater on the centers than on the petals of flowers ($F_{1,704} = 220.70, P<0.001$). There was a significant interaction between flower species and location of beetles on the center or petals of the flowers ($F_{2,704} = 100.91, P<0.001$; Fig. 3).

**Pollination benefits to flowers**

Beetles transitioned between flowers more frequently at higher temperatures ($F_{1,18} = 17.37, P<0.001$; Fig. 4).

**DISCUSSION**

**Heat capture by flowers**

We found evidence that flowers are able to capture heat, making them hotter than the ambient environment. The temperatures taken on the lily flower exceeded the ambient temperatures taken with the black body objects in both the sun and the shade. The structure of the lily flower, therefore, seems to be not only absorbing radiant energy from the sun, but magnifying it, making it hotter than its surroundings.

Additionally, in our intermittent temperature readings, we found that the center of the flower heats up faster than the petals across all three flower types. This suggests that the center of the flower magnifies radiant energy. The initial adaptive reason for this heat capturing mechanism is ambiguous. It might have been to provide thermal rewards to insect pollinators, or it might have evolved for an unrelated reason, such as to warm the flowers’ ovaries for faster gamete production. Regardless of its initial adaptive purpose, the ability of these flowers to capture heat at their center appears to have allowed them to develop a mutually beneficial relationship with insect pollinators, even though they did not coevolve.

**Beetle physiological benefits**

We found evidence that beetles benefit from increased temperatures. Beetle mating rates increase dramatically as temperature increases, suggesting that beetles benefit physiologically from extra heat. In the lab, increasing temperature by just four degrees nearly doubled copulation rates. We regularly recorded temperature boosts in flower centers that were larger in magnitude than the artificial heat boost the beetles received in the lab, the largest being a difference of 7.6 {\degree}C in the center of a lily in full sun. The temperature differences provided by flowers in sunlight are large enough that they likely affect beetle physiologically, thus providing a benefit to the beetles.

However, our experiment assumes that increased mating is beneficial to the beetle population as a whole. If mating in the wild involves males swarming females, as they did in the laboratory, it might not be completely beneficial. After the swarming, females might not have enough energy to lay eggs or gather enough food. For example, in the field we observed multiple instances of males hanging off the backs of females while the females tried to forage. These kinds of potentially negative impacts from mating might result in lower fitness of the females or their young. Future studies should investigate whether there is competing selection between the sexes of this beetle species.

While more experimentation needs to be done to determine that heat affects other beetle behaviors such as pollen foraging, the results of our lab experiment suggest that the beetles’ metabolism increases with temperature. Field
observations confirm that the beetles not only mate more in sunlight but also crawl faster and collect pollen more vigorously under sunny conditions. It seems probable that thermal rewards from flowers would benefit beetles in ways other than increased mating rates.

**Beetle heat preference**
Beetle abundance on all three species of flowers increased with temperature, which suggests that beetles will preferentially select warmer flowers. Additionally, beetle abundance depended on flower species; beetles were least abundant on dahlias and most abundant on lilies. These results, combined with the fact that dahlias seem to offer no thermal rewards, while lilies do, suggests that beetles prefer lilies over dahlias, possibly as a result of temperature. The combination of these findings suggests that beetles seek out thermal rewards from these nonnative plants.

**Pollination benefits to flowers**
Our observations of beetle movement between flowers confirmed that thermal rewards also benefit the flowers. The transition study demonstrated that, in the presence of ample sunlight, the beetles move more often between individual flowers (as opposed to simply basking in the center of a single flower to soak up warmth). Increased beetle movement creates potential for increased pollination, suggesting that beetles do, in fact, benefit the flowers. To provide an alternate line of evidence that flowers benefit from the presence of beetles, a future study might examine the effect of temperature on beetle pollen feeding rates.

Our four postulates were supported by the data we collected. Flowers (especially lilies) offer thermal rewards, beetles benefit from heat, beetles seek out heat, and flowers benefit from the presence of the beetles. The relationship between the beetles and the flowers therefore seems to be mutually beneficial.

Our findings are important not only to understanding interactions between species, but also to understanding how species ranges change and how biodiversity is maintained. Janzen (1967) theorized that tropical mountains pose larger physiological barriers to dispersal than temperate mountains because tropical species have narrower elevational range sizes. This phenomenon might boost tropical biodiversity by allowing allopatric speciation on either side of mountains. However, if insects in places that get cold (such as Cuerici) are given access through human intervention to newly available pockets of extra heat, they might be able to survive better at higher altitudes than before, thus lessening the physiological barrier. This will potentially allow them to move into new areas or increase in numbers, influencing entire community structures.

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**AUTHOR CONTRIBUTIONS**
All authors contributed equally.

**LITERATURE CITED**
NO TROUT ABOUT IT: RAINBOW TROUT (Onchorhynchus mykiss) SCHOOL SIZE AND PREDATION RISK AFFECT FORAGING BEHAVIOR

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Faculty Editor: Hannah ter Hofstede

Abstract: Effectively balancing the trade-offs between foraging and predator avoidance is vital to maximizing fitness for a species. Foraging might increase an organism’s risk of predation, but is necessary to ensure growth and development. Some organisms reduce predation risk by foraging in groups. Schooling in ocean- and lake-going fish is an example of such a defense mechanism. In this study, we investigated the effects of predator presence on schooling and foraging behavior in juvenile rainbow trout. At the Cuericí Biological Station, we introduced different numbers of farmed juvenile rainbow trout (1, 5, 10, or 20) into an experimental arena containing a food reward and either no predation risk or a large rainbow trout predator. We recorded the time it took for an individual fish to successfully feed, defined as feeding latency. Our results indicate that, in the absence of a predator, juvenile trout feed more quickly when they are in larger groups. However, in the presence of a predator, vigilance for predation risks seems to take priority, as increasing school size has no effect on feeding latency. These findings suggest schooling behavior, such as in juvenile rainbow trout, might vary in different situations to more effectively balance the tradeoff between foraging efficiency and vigilance.

Key words: feeding latency, fry, school dynamics, vigilance

INTRODUCTION
Organisms must balance resource tradeoffs among three objectives to maximize reproductive fitness: foraging, avoiding predation, and finding a mate. Before sexual maturity, or outside of breeding season, an organism must prioritize the trade-offs between foraging and predation avoidance. If an organism spends more time foraging, it takes advantage of more opportunities to grow. However, if the same organism does not divert enough time towards vigilance for predation risks, it might be consumed and lose its reproduction potential. Numerous species use social defenses as methods to avoid predation. For example, if many organisms gather in one area, the chance that one individual will be targeted is minimized given the prey-to-predator ratio. This dilution effect has been observed in many species, such as populations of mayflies that emerge synchronously in large groups, as well as in aggregations of mud-puddling butterflies (Alcock 2013).

For young fish, the risk of predation is immensely high; it is common for them to be consumed by almost any organism of equal or larger size, including conspecifics. To survive past this vulnerable life stage, many ocean- and lake-going fish species have converged on schooling behavior as a defensive strategy. One explanation for schooling is the selfish herd theory, under which aggregations form when all individuals in a population attempt to place themselves behind another conspecific to better avoid predation risk. (Alcock 2013). If a large group of fish concentrates in a single mass, the probability that a predator will find the group, let alone strike at a given individual, decreases dramatically. If a predator does locate the school, the schooling dynamic can disorient the attacker and enable the school’s members to successfully escape.

Juveniles of many solitary fish employ schooling as defensive tactic until they can survive on their own, including species such as rainbow trout (Onchorhynchus mykiss). Rainbow trout lay all of their eggs in a nest (redd) and the eggs hatch at the same time. When the juveniles (fry) emerge from the substrate, they school together to avoid predation. Characterizing schooling behaviors in fry is important for understanding rainbow trout foraging and survivorship in both natural and farmed settings.

In this study, we investigate how the schooling dynamics of rainbow trout fry and the presence of a predator affect risk-taking behavior.
while foraging. If the trade-offs between predator avoidance and foraging success affect fish activity, then schooling should change fry behavior by enabling greater collective vigilance towards predation risks. Thus, in the presence of a predator, juveniles should invest more time in vigilance toward predation risks and less time in foraging. Yet, with an increase in school size, we expect an increase in the group’s collective vigilance to encourage the foraging behavior of all individuals, even in the presence of a predation risk.

METHODS

Field Experiment
All experimental procedures were conducted in a 9.5-meter cement trough built by rainbow trout farmer Don Carlos at Cuericí Biological Field Station in Costa Rica. We used Don Carlos’ farmed fry as our trial subjects, and we used his adult rainbow trout as our experimental predators. All of the fish were habituated to eating commercial fish food instead of their natural diet (insects, small fish, etc.). For average length measurements, we randomly selected and measured 10 fry (5.85 ± 0.28 cm) and 7 adults (37.86 ± 2.29 cm).

On 25-26 January 2017, we recorded the foraging and schooling behavior of juvenile rainbow trout presented with food. To create our experimental arena, we placed mesh partitions in the trough 2.58 meters apart from each other (Figure 1). The water for the trough came from a natural spring and flowed through the full setup. We classified two halves of the experimental arena according to proximity to the food reward (near and far). We dropped fry in sequential trials of 1, 5, 10, or 20 individuals adjacent to the partition on the far half of the arena and placed the food source on the partition at the other end. This setup allowed small food particles to sink throughout the trial; the captive trout prefer to eat food as it sinks and tend to ignore food once it is on the ground. We measured the time it took for an individual fry to feed after being dropped into the experimental arena (defined as feeding latency). We stopped the replicate if a fish did not successfully feed within five minutes. We also measured how long the fry spent in each half of the experimental arena (near or far).

We used different fry for every trial and replicate to account for the probability that some individuals feed more quickly than average across a distribution of all of the fish. Tested fry were stored outside of the near end partition (in the fry storage area). We covered the mesh partition separating the storage area from the experimental arena with a wooden board and cooler lid so the fry in the experimental setup would not be affected by the schooling of the already-tested fish.

For the predator trials, we used the same experimental design and added an adult rainbow trout to the experimental arena with the juveniles. To randomize the effect of the adult on the juvenile, the adult was switched after each trial.

Figure 1. A schematic depicting the experimental setup. Dark gray bars indicate mesh partitions in the trough. Water flow was directed towards the fry (from right to left, from the source flow area to the fry storage area).
**Statistical Analyses**

Using JMP Pro V. 13.0, we predicted feeding latency with a generalized linear mixed model that included predation risk (categorical variable, fixed effect), school size (continuous variable), the interaction between predation risk and school size, and predator identity (categorical variable, random effect). We then conducted post-hoc regressions to analyze the effect of school size in each predation condition. Finally, we used a two-way ANOVA to predict the relative amount of time that each school size spent in either the near or far side of the experimental arena, depending on predation risk.

**RESULTS**

The interaction between number of fish and presence of predator significantly affected feeding latency ($F_{1,43} = 26.20, P < 0.01$). In the absence of predation risk, larger schools took less time to feed than did smaller schools (slope = -14.01 ± 1.15, $P < 0.001$) but this relationship was nonexistent in the presence of a predator (slope = -3.33 ± 1.86, $P = 0.10$; Fig. 2). The individual predator in the trial did not significantly affect feeding latency (95% CI = -617.32, 779.59).

**Behavioral Observations**

Across all non-predator trials, fry approached and inspected the food numerous times before feeding. However, the frequency of inspections prior to striking the food was lower when school size was 20 individuals than when it was 10 individuals. The shortest feeding latency recorded ($t = 14s$), from a school of 20 fry, appeared to be close to the fastest time possible for a given individual to orient itself and swim to the food after being dropped into the experimental arena.

Across the predator trials, the adult rainbow trout never struck out at the fry, yet the fry always fled from the adult when it swam towards them. For most trials, the adult remained near the food source, which challenged the fry to balance vigilance for predation with foraging benefits. When the adult remained stationary, the fry school regularly approached and swam very close to the adult. Yet, groups that approached the adult did not appear to then strike at the nearby food source any more quickly than groups that always stayed farther away from the adult. During three trials, the adult trout swam around the experimental arena instead of remaining stationary, which coincided with the fry apparently fleeing from its path.

**DISCUSSION**

In the absence of predation risk, we found that schooling resulted in individual juvenile rainbow trout retrieving a food reward more quickly than if they were alone. Across non-predator trials, there was no significant difference in feeding latency between an individual fry and a school of 5 individuals, although the 5-fish schools generally fed more quickly. Surprisingly, individual fish failed to retrieve the food reward in the 5-minute time limit in all but one trial. However, feeding latency decreased with each school size larger than 5 individuals. Though it is possible that a larger school has a higher chance of detecting a food source more quickly than an individual, we observed individual fry inspecting the food source...
numerous times without feeding when in a large school. Rather, schooling seems to play an influential role in the ability of fry to prioritize foraging over vigilance when predation risks are not present.

However, when a predator risk is present, the observed effect of a school changes. Instead, the school seems to take on a purely defensive role in minimizing the risk of predation. There was no significant difference in feeding latency among school sizes in the predator trials, suggesting that the threat of a predator overrides the reduced vigilance observed in larger groups. Perhaps this priority of vigilance over foraging is a consequence of differing selection pressures for prey and predators. In the wild, juvenile trout must avoid potential predators (i.e., any organisms larger than themselves) for their own survival, so avoiding adult trout at the expense of a foraging opportunity would be innate. Since predators might eat a variety of prey items, adult trout habituated to eating commercial fish food might not experience the same level of selective pressure to attack fry (a novel food source) as fry do to avoid the adults. Thus, we were able to sufficiently analyze the effect of predation risk on fry foraging behavior, even if this risk was not real in a farmed setting. While schooling might offer a sense of safety to individual fish when a predation risk is both present and absent, schooling only offers an additional impetus for foraging behavior when imminent predation risks are diminished.

In rainbow trout fry, feeding latency is affected by both school size and the presence of predation risks. There is the possibility that, in a population of fish, some will simply choose to forage more quickly than others. Yet, if such were the case, increasing school size would simply allow a greater chance for one of these individuals to produce a lower feeding latency for the group as a whole. We did not re-sample the fry that struck the food first in a given trial to determine whether these individuals simply exhibited a consistent prioritization of foraging over vigilance. However, if this pattern of behavior were apparent in a given subset of individuals, we would not expect to have seen the observed, consistent pattern of school size affecting foraging latency. In addition, while the swimming activity of the adult trout during predation risk trials was unpredictable, we were able to account for this activity by including individual predators in our generalized linear mixed model. Thus, by switching the predator used for each trial, we were able to control for the effects of predator activity within the model. Even with the limitations in the number of trial series that we were able to produce in the given study period (10 no-predator trials, 5 predator trials), our results show significant trends in fry schooling behavior with regards to predation risk in the surrounding environment.

Future studies could explore additional effects of fry age class and predator swimming behavior on feeding latency. Our study only focused on one size class of juvenile trout. Replicating our investigation with several different fry size classes could determine whether an increase in size class correlates with a lower feeding latency, with or without a predation risk. Furthermore, studying the effect of the relative time that the predator stays on each side of the tank (far, near) in relation to school size and feeding latency could indicate whether there is a safe distance at which there is an effect of group size on feeding latency.

Apparently, schooling in rainbow trout fry does not serve as a purely defensive behavior. Depending on whether an imminent threat is detectable, schooling might also facilitate foraging behavior in individuals. As previously stated, schooling is generally considered under the selfish herd theory to be a defensive tactic that reduces individual vulnerability to predators. While this hypothesis might offer another explanation of schooling behavior among rainbow trout fry, given our results, the utility of the school might extend beyond security to benefits in foraging. Grouping in populations cannot only be characterized by a single purpose, such as defense or foraging advantage. Rather, like rainbow trout fry schooling, grouping behaviors might serve different functions and manifest differently depending on the external factors at play in the environment.

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AUTHOR CONTRIBUTIONS
All authors contributed equally to the experimental design, data collection, data analyses, and the production of this report.

LITERATURE CITED

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Abstract: Flight is a method of locomotion that has evolved convergently in many species. Birds have evolved a variety of morphologies, producing large differences between species in both flight mechanics and energetic costs. Bats are the only mammals to have evolved a true form of flight and, like birds, display great diversity of wing morphology. Within the genus Pteronotus, two species have wings that extend entirely across the back and meet at the spine, designated here as a fused wing morphology. In this study, we compared wing morphology and echolocation call measurements that correlate with maneuverability between P. gymnonotus, with fused wings, and P. personatus, with wings that meet along its sides. We sought to determine whether the fused wing morphology of P. gymnonotus might produce differences in flight performance from related species that do not have this adaptation. The wing loading and aspect ratio of a flying organism enable us to make inferences about the speed, maneuverability, and energetic costs of its flight. We therefore measured wingspan, wing surface area, and body mass of both P. gymnonotus and P. personatus to calculate the wing loading and aspect ratio of each species. We compared these values across other species in the Mormoopidae family and compared the wingspan of P. gymnonotus to other bat species of similar mass. We further analyzed the call frequency of both species as a proxy for maneuverability potential. We did not find evidence that the fused-wing morphology of P. gymnonotus improves its maneuverability in flight. Thus, we cannot conclude whether the morphology of P. gymnonotus exhibits outstanding qualities that would give this bat more maneuverable flight.

Key words: Adaptation, echolocation, flight mechanics

INTRODUCTION
Many species have converged on flight as a method of locomotion. Birds, bats, and many insects have independently developed methods of flight. All of these organisms have structural constraints that limit the energetics and mechanics of flight. There are two standardized measurements that dictate flight cost and efficiency: aspect ratio and wing loading. Aspect ratio influences drag and is calculated as the square of the wing span divided by the surface area of the wing. Wings with low aspect ratio produce flight that is energetically costly but maneuverable, while wings with high aspect ratio produce flight that is inexpensive yet offers less control. Wing loading, the extent to which the wings support the mass of the organism, is calculated as the weight of the organism over the wing surface area. Organisms with high wing loading are capable of high speeds but generally have low maneuverability, while the reverse is true for low wing loading. Therefore, low aspect ratio and low wing loading are crucial to maneuverability.

For bats, there is a trade-off between maximizing maneuverability and conserving energy in flight. Bats vary greatly in their morphology and echolocation calls. These calls change based on the bat’s ability to maneuver through its environment. Higher-pitched echolocation calls attenuate faster but provide more detailed information about a small area, while lower frequency calls return less detailed information about a larger area. Bats that can move through cluttered environments generally do not need low frequency calls because they do not require spatial information from a far distance. Instead, they employ higher pitched echolocation calls because their flight requires greater acuity; to avoid clutter in their surroundings, more maneuverable bats must be able to perceive finer details undetectable by lower frequency echolocation.
Outside of the El Campanario Research Station on the Osa Peninsula, Costa Rica, there is a shoreline cave containing three species of bats of the family Mormoopidae. One of the bats, *Pteronotus gymnonotus*, is dubbed the naked-backed bat because its wings extend across its back instead of ending along its side like the other species in the *Pteronotus* genus (Figure 1). Although the skin of each wing of *P. gymnonotus* extends to the centerline of the back, the skin is only attached at this centerline. Thus, *P. gymnonotus* still has fur on its back, but it is covered by the wingskin. This structure produces a pocket under *P. gymnonotus*’ wing. While the wings of *P. gymnonotus* extend over the arch of its back, the wings of most other bats meet at the lateral midline of its body. Since *P. gymnonotus* is one of only two bat species, both in the genus *Pteronotus*, with this morphological feature, it is reasonable to assume that the fused wing is an adaptation that has diverged from a common ancestry. One hypothesized explanation for this adaptation is that it is useful for wicking off rain mid-flight (Aitkin N., pers. comm.). However, this “umbrella hypothesis” does not explain why these rare bats are found in the seasonally dry rainforest of the Osa peninsula, nor does it explain why only two bat species (the other being *P. davyi*) in the genus *Pteronotus* exhibit the “fused wing” adaptation, whereas other bats do not.

An alternative explanation for the origin of *P. gymnonotus*’ wing morphology focuses on the principles of flight mechanics. We tested the hypothesis that the fused wing morphology of *P. gymnonotus* provides greater maneuverability. Stunt planes and high performance fighter jets are built with a single wing extending across the top of the fuselage, which allows for more maneuverability and control, whereas commercial aircraft have wings extending from the centerline. Based on these designs, we predicted that *P. gymnonotus* would be more maneuverable than other bats. Thus, this species should have a lower aspect ratio and wing loading, and should call with higher frequencies to prioritize information pertinent to its immediate surroundings.

**METHODS**

**Field Methods**

During the nights from January 31 - February 2, we collected data on bats in a cave near the El Campanario Biological Research Station on the Osa Peninsula in Costa Rica. To determine whether *P. gymnonotus* is more maneuverable than other species in *Pteronotus*, we investigated morphological differences between *P. gymnonotus* and *P. personatus*. We used two separate techniques to capture the bats for our study: mosquito netting and mist netting. To catch the bats with mosquito nets, two researchers stood across an area with high bat traffic and stretched a mosquito net between them. The two researchers waited until a bat flew into the net, at which point they folded the net in half to capture the bat. We also set up mist nets along a trail about 100 m from the bat cave and waited for bats to fly into the nets and become ensnared. Bats were stored in cloth bags for transport from the field to the lab.

**Wing and Echolocation Call Measurements**

To obtain the weight of each bat, we weighed the bat and the bag together, then weighed the bag alone and took the difference between the two values. To measure wingspan and surface area, we traced the outline of the bats’ wings and bodies on 0.16 cm² graph paper. We measured the wingspan of each bat and counted the number of squares outlined by the trace to get the wing surface area. We calculated wing loading by dividing the mass of each bat by its wing surface area, and we calculated the aspect ratio by dividing the squared...
wingspan by the wing surface area. Bats were then replaced in the cloth bags and were assigned a number to keep track of each individual for frequency measurements during their release.

To determine whether *P. gymnonotus* calls with a higher frequency, we built a bat tunnel to direct their flight and make it easier to measure their calls. To build the tunnel, we tied a rope between two trees about 1 meter off the ground. We then draped two bedsheets over the line and a mosquito net along the bottom to create a tent-like structure to funnel the bats’ flight. We released the bats at one end and one researcher sat with a recording tablet at the other end. We used Avisoft recorder software on a Windows tablet and a microphone tuned to ultrasonic frequencies (40-120 kHz) to record their calls. After recording each bat’s echolocation calls in the field, we used Avisoft SASLab Lite to measure the peak frequency (frequency with the most energy in kHz) of each harmonic within the call.

**Statistical Analyses**

We ran all statistical analyses in JMP Pro V 12. We conducted 2 two-sample t-tests to test for differences between bat species in wing loading and aspect ratio. We plotted wing loading and aspect ratio of *P. gymnonotus* together with known values of other species in the Mormoopidae family (Norberg and Rayner 1987). We also plotted our measured average wingspan of *P. gymnonotus* against tabulated wingspans of similarly massed bats from numerous families (Norberg and Rayner 1987). Thus, we could determine whether the wingspan of *P. gymnonotus* is small for its size, as a proxy for greater maneuverability. For the frequency analysis, we conducted a two-sample t-test comparing each of three harmonics in the calls between *P. gymnonotus* and *P. personatus*.

**RESULTS**

**Wing Morphology**

We captured 13 *P. gymnonotus* (12 female, 1 male) and 6 *P. personatus* (5 female, 1 male) during our study. To standardize for sexual dimorphism, we only analyzed the data collected for the females of each species. One female *P. personatus* was pregnant and was excluded from measurements of mass, wing surface area, and wingspan to avoid bodily injury. Thus, all morphological results were developed from a sample size of \( N = 12 \) *P. gymnonotus* and \( N = 4 \) *P. personatus*.

The aspect ratio of *P. gymnonotus* was significantly lower than that of *P. personatus* (paired-\( t = 2.81, P = 0.014, df = 14; \) Figure 2). However, the wing loading of *P. gymnonotus* was significantly higher than that of *P. personatus* (paired-\( t = -2.43, P = 0.029, df = 14 \)). When comparing wing loading and aspect ratio to other species in Mormoopidae, *P. gymnonotus* fell close to *P. davyi* (the only other naked-backed Mormoopidae species), in between *P. personatus* and *M. megalophylla* (Figure 3). In a broader comparison of wingspan in bat species with similar average weights, *P. gymnonotus* fell near the center of the distribution (\( N = 27 \) species, mass range from 0.0125 - 0.0176 g; Figure 4).
Echolocation Call Frequencies

We collected call frequency data for all twelve female *P. gymnonotus* and four female *P. personatus*. We tried to collect frequency data on the pregnant *P. personatus* individual, but she escaped from the entrance of the tunnel and was not recorded. Thus, the sample size and identity of the bats of both *P. gymnonotus* and *P. personatus* were the same across morphological and call frequency analyses.

*P. personatus* appeared to consistently invest the majority of its echolocation energy in the second harmonic of its call, manifested in a higher amplitude relative to other harmonics in decibels. On the other hand, *P. gymnonotus* seemed equally likely to invest more energy in either the second or third harmonic of its call. Therefore, comparisons in frequency were made within each harmonic rather than within a single, dominant harmonic. *P. gymnonotus* exhibited a lower peak frequency than *P. personatus* within the first, second, and third harmonics of its call ($F_{1,52} = 6593.47, P < 0.001$ for harmonic 1, $F_{1,52} = 4228.36, P < 0.001$ for harmonic 2, $F_{1,52} = 897.47, P < 0.001$ for harmonic 3; Figure 5).
Behavioral Observations
In all 13 call frequency trials (across males and females), \( P. \) gymnonotus flew through the tunnel after release. Conversely, in three of six total \( P. \) personatus trials, \( P. \) personatus flew away from rather than through the tunnel. Furthermore, we caught \( P. \) gymnonotus only in the mist net positioned on the public trail near the cave. In contrast, we caught only one \( P. \) personatus in the mist net, while the rest were caught in the mosquito nets positioned directly adjacent to the cave entrance. We captured only a very small subset of the bats observed leaving the cave; most flew over or around our nets upon approach.

DISCUSSION
This study did not produce compelling evidence that \( P. \) gymnonotus’ wing morphology improves its maneuverability in flight. If a constant wing across the back offered greater maneuverability to \( P. \) gymnonotus over other species within \( Pteronotus \), we would expect to see a lower aspect ratio and wing loading than in \( P. \) personatus. Although \( P. \) gymnonotus has a lower aspect ratio than \( P. \) personatus, its relatively high wing loading is not consistent with the predicted results, had it evolved to be more maneuverable than \( P. \) personatus. The body dimension data provide conflicting lines of evidence regarding maneuverability. It is unclear whether \( P. \) gymnonotus or \( P. \) personatus is the more maneuverable bat. The data comparing \( P. \) gymnonotus’ and \( P. \) personatus’ respective wing structures to those of otherwise similar bats does not support the importance of the continuous wing for maneuverability in \( P. \) gymnonotus. When compared to the rest of its genus, \( P. \) gymnonotus does not have an unusual wing loading or aspect ratio, nor is its wingspan shorter than those of bats of similar weights. In fact, we found \( P. \) gymnonotus to be nearly average in both of these categories. These findings suggest that \( P. \) gymnonotus’ maneuverability is not exceptional among bats to which it is similar.

Furthermore, \( P. \) gymnonotus produced lower frequency echolocation calls than did \( P. \) personatus. These findings suggest that \( P. \) gymnonotus is better adapted for long distance echolocation, since lower frequency calls are better suited for environments that have fewer obstacles and require lower flight maneuverability. Although this was not consistent with our predictions, we also found that \( P. \) gymnonotus were almost twice as heavy as \( P. \) personatus. Because larger bats generally produce lower frequency calls, \( P. \) gymnonotus might not be directly comparable to \( P. \) personatus. Due to the limited time and equipment available, we were not able to conduct any further experiments on frequency and flight. More research is needed to investigate these differences in calls and how they relate to the flight mechanics of both species. In the context of our broader results, these echolocation findings are inconclusive. Although consistent with the wing loading results, they imply that \( P. \) gymnonotus might be more adapted to speed and therefore do not provide conclusive information about maneuverability.

We observed behavioral differences that could provide support for the superior maneuverability of \( P. \) gymnonotus. Although not conclusive, the finding that \( P. \) gymnonotus flew through the tunnel more readily than \( P. \) personatus suggests that \( P. \) gymnonotus might be better prepared for flying through confined spaces and thus might be more maneuverable than \( P. \) personatus.

It is challenging to make comparisons between the relative importance of wing loading or aspect ratio and maneuverability without empirical data on flight patterns. More conclusive results exposing the function of the fused wing in \( P. \) gymnonotus could be achieved through future studies directly comparing \( P. \) gymnonotus’ and \( P. \) personatus’ maneuverability in flight tests. Such tests could involve obstacle courses that would make it possible to more effectively compare the bat species’ relative agility in flight. Alternatively, using a wind tunnel to assess the aerodynamics of each species would offer another method of directly measuring the effects of wing morphology on flight maneuverability. This method is particularly suited to investigating whether the pocket observed under \( P. \) gymnonotus’ wing serves an aerodynamic purpose. Our study provides evidence that there are significant differences in wing morphology that might affect flight mechanics in bats, but future research could show how this fused wing changes flight within the \( Pteronotus \) genus.

In conclusion, these ambiguous results emphasize the difficulty of using anatomy to deduce function. Wing anatomy cannot be used as
a proxy for maneuverability because it is important for many other aspects of bat flight. It is unusual for an anatomical feature to evolve exclusively in response to one pressure. Due to the tradeoffs associated with flight, those characteristics important to maneuverability are likely to be obscured by the characteristics important to other aspects of flight. As a result, no single measurement (such as wing loading or aspect ratio) is entirely representative of maneuverability, and multiple measurements are likely to produce conflicting conclusions. Although these trade-offs add complexity to the study of flight, it is in balancing these trade-offs that organisms are able to effectively adapt to their aerial niche. It is therefore through the understanding of the trade-offs contributing to flight evolution that we will be able to understand the functional significance of morphological features.

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AUTHOR CONTRIBUTIONS
All authors contributed equally to the experimental design, data collection, data analyses, and the production of this report.

LITERATURE CITED
Abstract: Intraspecific niche differentiation can structure spatial and temporal distributions of a species within an ecosystem, especially when niches shift as individuals grow. These niches might be the result of multiple ecological or physiological factors, such as intraspecific competition or differences in the physiology between sizes. At El Campanario Biological Station, small terrestrial hermit crabs (family Coenobitidae) were observed on the beach during the day and large terrestrial hermit crabs on the beach during the night. We examined the roles of competition and thermoregulation on intraspecific niche differentiation for these hermit crabs. We surveyed the densities and average sizes of hermit crabs in the beach and adjacent leaf litter, both at night and during the day, to determine the spatial and temporal distribution. Additionally, we conducted habitat preference trials to test for the effects of competition on crab distribution, and we measured crab temperatures on both substrates during the day and night to examine the role of thermoregulation in determining their distribution. We found that crab densities were greater in the leaf litter than the sand regardless of time of day. Average crab size varied with time of day and substrate type, with the smallest crabs on the beach during the day and the largest crabs on the beach at night. However, according to the preference tests, all crabs appear to prefer litter. There was no evidence of competition between crabs, but heat tolerance seemed to differ between hermit crab size classes, suggesting that behavioral thermoregulation plays a primary role in their spatial and temporal distribution.

Key words: niche differentiation, size classes, spatio-temporal distribution, terrestrial hermit crabs

INTRODUCTION
Both inter- and intraspecific niche differentiation can structure spatial and temporal distribution patterns of organisms within an ecological community. With interspecific niche differentiation, competing species use an environment differently, allowing them to coexist. With intraspecific niche differentiation, subpopulations of the same species use environmental resources differently. The niches of many species change with the ontogeny of organisms. Factors like intraspecific competition or differences in physiology might lead to differing spatial and temporal distributions of large and small organisms of a given species. Observations suggest that these factors might apply to terrestrial hermit crabs living on the border of beach and forest habitats.

In Costa Rica, smaller hermit crabs have been observed on the beach during the day and larger crabs are seen on the beach primarily at night, implying systematic daily movements between habitats. This apparent niche differentiation between size classes might be the result of competition, in which larger crabs might displace smaller crabs from a limited space in the leaf litter habitat between the beach and adjacent forest, leading to different spatial distributions of crab sizes. Alternatively, competition might not structure the spatial distributions of the hermit crabs. Niche differentiation might also be the result of differing thermoregulatory abilities of differently sized crabs, as smaller crabs might be able to dissipate heat more efficiently than larger crabs, and thus can remain on the beach during the day, while the large crabs must remain in the refuge of the leaves. Alternatively, thermoregulation might play no role in the niche differentiation of the crabs.

METHODS
Density and shell size
To quantify the spatio-temporal distribution of hermit crabs, we surveyed hermit crab density on sand and nearby leaf litter substrates on the beach of El Campanario Biological Station during the day and night. We established plots around existent aggregations of hermit crabs that were at least 1 square meter in area. In each plot of hermit crabs, we measured plot area and each individual’s shell length (from the base of the aperture to the apex) and opening diameter (from the base to the...
top of the aperture) as proxies for crab body size. We performed two such surveys on each substrate type twice daily during low tide on 31 January and 1 February, 2017.

To calculate hermit crab density, we divided the number of hermit crabs by the plot area. We used a square root transformation to normalize the distributions of the density and size data. We used a two-way ANOVA to test the effects of substrate, time of day, and substrate*time of day on hermit crab density. We used another two-way ANOVA to test the effects of substrate, time of day, and substrate*time of day on average hermit crab size.

**Competition and substrate preference**

To determine whether competition over habitat space affected the distribution of hermit crab size classes, we first tested the habitat preferences of each size class in the absence of the other. We constructed a 0.5 m x 0.25 m arena filled with sand and covered half of its surface area with leaf litter collected from the beach at the station. Hermit crab size classes were chosen to reflect the extremes of the size distribution of the sampled crabs. For both large (> 25.5 mm) and small (< 13.7 mm) size classes, we placed six hermit crabs in the arena in one of four starting arrangements: all on sand, all in litter, all in the middle, or distributed evenly across sand, middle, and litter. This eliminated the bias of starting position. For each trial, we waited three minutes before recording the crabs’ end positions as a proxy for habitat preference. For both size classes, we performed this procedure twice during the day and twice during the night. We used chi-square tests to quantify the habitat preference of both size classes.

We repeated this protocol using three small and three large individuals in each trial to evaluate whether the presence of the other size class affected end position. We then halved the area of the arena to restrict habitat space and repeated the mixed size class trials. We used chi-square tests to determine whether the frequencies of end positions for either size class were affected by the presence of the other size class.

**Thermoregulation**

To examine the thermal differences between sand and leaf litter substrates, we recorded hermit crab temperatures *in situ*. We used a thermocouple (Bat 12 probe) to measure the temperature inside shells of randomly selected hermit crabs on both sand and litter substrates during the day and at night. We tested the distribution of our data and found that it did not fit a normal distribution. We performed an inverse transformation on the distribution of crab temperature to enhance normality. With the assumption that a general linear model would be robust to the remaining deviation from a normal distribution, we tested the effects of substrate type, time of day, crab size, time of day*substrate, time of day*size, substrate*size, and time of day*substrate*size on crab temperature.

We experimentally compared the heating rate of large and small crabs in the sand during the day. We placed three crabs of both size classes on the sun-exposed sand and recorded their temperatures every twenty seconds for 100 seconds. We used a general linear model to test the effect of hermit crab size class on heating rate.

In the course of our trials, we kept small and large hermit crabs to observe overnight behaviors, such as burrowing.

**RESULTS**

**Density and shell size**

Hermit crab density was significantly greater in leaf litter than on sand ($F_{4,11} = 31.48, P < 0.001$), but density was not affected by time of day ($F_{4,11} = 0.71, P = 0.42$; Fig. 1). Shell length and opening diameter were strongly positively correlated (slope = 1.11 ± 0.03, $P < .001$, $r^2 = 0.67$), so we used opening diameter as a proxy for crab body size. We considered this measurement a more accurate descriptor of body size because opening diameter restricts the size of the crab that can enter the shell, whereas shell morphology might affect shell length without reflecting body size. There was a significant interaction between substrate and time of day ($F_{3,11} = 89.31, P < 0.001$); the difference in average size between day and night was higher for crabs on the sand than in leaf litter. A post-hoc Tukey’s HSD with an alpha value of 0.05 showed significant differences between each combination of factor levels. Crabs on the sand at night were largest, followed by crabs in the litter at night, crabs in the litter during the day, and crabs on the sand during the day (Fig. 2).
Competition and substrate preference

When tested in an experimental arena, hermit crabs more frequently chose the leaf litter substrate (84.58%) than the middle (7.86%) or sand (7.56%) regardless of starting position (chi-square = 6.39, $P = 0.17$, df = 4) or size class (chi-square = 0.87, $P = 0.65$, df = 2). This pattern was maintained for both small (chi-square = 0.05, $P = 0.49$, df = 2) and large crabs (chi-square = 2.97, $P = 0.11$, df = 2; Fig. 3) in the presence of the opposite size class.

Thermoregulation

There was a significant interaction between substrate and time of day (chi-square = 12.64, $P < 0.001$, df = 1; Fig. 4), such that the effect of substrate on in situ temperature was higher during the day than during the night. Due to the interaction effect, we ran two post-hoc t-tests to investigate the effect of substrate on temperature for each time of day. Due to running three tests on the same data, we used a Bonferroni correction to adjust the alpha value from 0.05 to 0.017. Post-hoc t-tests showed that crab temperatures were significantly different on the sand and in the litter during the day ($t_{10.1} = 6.18$, $P < 0.001$), but they were not significantly different on the sand and in the litter at night ($t_{10.1} = 2.03$, $P = 0.059$). The interaction of time of day and crab size was nearly significant, suggesting that crab size might be negatively related to crab temperature during the day (chi-square = 3.56, $P = 0.059$, df = 1; Fig. 5).

We found that heating rate and crab size were unrelated ($F_{1,4} = 4.00$, $P = 0.12$; Fig. 6). However,
we observed that heat negatively affected the large crabs more than the small crabs. During the trials, both sized hermit crabs retreated into their shells. After the trials, large individuals took a couple of minutes to re-emerge from their shells, whereas small hermit crabs emerged almost immediately, even though both size classes reached about 35°C by the end of the two-minute period.

DISCUSSION

The results of our density and size surveys support the observed pattern of primarily small crabs on the beach during the day and primarily large crabs on the beach at night. The higher density of crabs in the leaf litter throughout the day could be because the leaf litter habitat provides refuge from the sun, making the litter a preferable habitat for all hermit crabs. The mean crab sizes were smallest on the sand during the day, largest on the sand at night, and intermediate in the litter during all times of day. These spatio-temporal variations in mean shell size suggest a substantial movement between litter and sand environments for different crab sizes classes over a twenty-four hour cycle.

Our results did not support competition as a cause of the spatial and temporal distribution of crab sizes. Both small and large crabs preferred the leaf litter habitat during the preference trials. If competition were present, the larger crabs would have displaced the smaller crabs when both were put in a space-limited litter habitat. It is possible that, given the stress of the experimental setup, the crabs might have been scared into hiding in the leaf litter, or we might not have limited the space enough to demonstrate competition. However, since the density of crabs was higher in the litter than on the beach during both the day and night, the crabs’ demonstrated preference for leaf litter habitats was likely not an artifact of our experimental setup. Leaf litter did not appear to be saturated with individuals, which further discredits competition for space as a primary cause of habitat stratification by size class.

Based on our results, thermoregulation offers the strongest explanation for the observed spatio-temporal distribution of crab sizes over the course of a day. Overall, smaller crabs were warmer in their natural environments compared to larger crabs, but their rates of heating were not...
significantly different. All the crabs we measured were warmer than their ideal temperature range for feeding (24-28°C; Vosjoli 2005). We observed that the larger crabs seemed to have a more negative reaction to the heat, which suggests the threshold for dangerously high temperatures might be lower for large crabs than for small crabs. In addition, we found that in situ crab temperatures are determined by an interaction of time of day and substrate type; the temperatures of crabs in sand change more over the course of a day than the temperatures of crabs in leaf litter. The difference in temperature between leaf litter and sand is also much smaller during the night.

Though the interaction of time of day and crab size was not significant, there was a trend toward a negative correlation between crab size and temperature during the day, suggesting that, during the day the bigger crabs were cooler than the smaller crabs. Larger crabs might have a lower tolerance for heat than smaller crabs because of their smaller surface area to volume ratio. Thus, they might behaviorally thermoregulate by moving to areas where temperature is lower. This could explain why large crabs seem to avoid going onto the beach during the day.

We observed larger hermit crabs burrowing into the sand overnight, while the smaller crabs remained on the surface of the sand. This burrowing behavior might be prevalent in the larger crabs to aid in thermoregulation, dissipating heat in cooler sand when it is difficult to avoid the heat of the sun. It remains unclear where the larger crabs go during the day. Based on anecdotal evidence, we predict that they migrate into the forest, but they might also burrow, as demonstrated in the lab. Further investigation is required to determine their daily routine, and what prompts burrowing rather than migratory behaviors.

Several other potential explanations exist for the spatial and temporal distribution of hermit crab sizes. Smaller crabs might prioritize proximity to a water source because their higher surface area to volume ratio could leave them more vulnerable to desiccation than larger crabs. Smaller crabs’ ability to more efficiently dissipate heat might allow them to cross the hot sand during the day and access a source of water. The larger crabs might only move onto the sand to hydrate at night when the temperature is lower. According to Brodie (1998), terrestrial hermit crabs of different sizes travel at the same speed. However, larger crabs might expend less energy to travel the same distance. If small hermit crabs must obtain resources, such as water, from the sandy substrate, it might be too energetically costly to periodically migrate to the leaf litter for heat avoidance. In addition, hermit crabs are obligatorily social, as they need to exchange shells to further their development. The beach might serve as an aggregation ground, where large hermit crabs can gather for large shells at night, and small hermit crabs can gather for small shells during the day. Stochastic aggregation might drive the temporally stratified size distribution of hermit crabs in this boundary habitat. Another explanation for the observed distribution is that large hermit crabs are more conspicuous targets for diurnal predators and might take refuge in the leaf litter.

Overall, differently sized terrestrial hermit crabs distribute themselves based on substrate and time of day, and this spatial and temporal distribution of size classes appears to be an example of intraspecific niche differentiation. Differing thermoregulatory abilities and the resulting behaviors between size classes appear to be a primary driver of intraspecific niche differentiation in this system, rather than intraspecific competition. Other factors depending on size variation within a population, such as predation and hydration, warrant future examination to better understand the observed spatial and temporal patterns of crab size classes. Populations like these terrestrial hermit crabs that regularly occupy multiple habitats within a short time interval might experience a variable set of resources and abiotic conditions, which allows groups of individuals within the population to use the habitat in different ways. Groups of individuals at varying developmental stages can alter their use of the same environment to maximize the potential of a given habitat. This partitioning might benefit individuals, as well as the whole population.

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AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED

SHELL CONSCIOUS HERMIT CRABS: *COENOBITA COMPRESSUS* RESPONSE TO SHELL RESOURCE LIMITATIONS

LOUISE J. BARIAS AND MARIKO C. WHITENACK

**Abstract:** Resource limitation can influence behavior in animals. The abundance and physical structure of shells might be a limiting resource for hermit crabs. Hermit crabs remodel shells once they are no longer being used by the gastropods that create them, and this is an energetically costly process. In large populations, hermit crabs have a greater shell market to choose from, suggesting that hermit crabs in larger populations should experience better shell fit. In addition to differences in size, there can be variation in shell shape. For hermit crabs, there might be a particular shell shape that confers optimal physical benefits, and hermit crabs might demonstrate a preference for this optimal shell shape. We studied hermit crab populations at two beaches on the Osa Peninsula in Costa Rica. We found that shell fit does not significantly vary between large and small populations of hermit crabs. There was no significant difference in crab locomotion speed for spherical and conical shells, and hermit crabs did not prefer one shell shape over another. Larger hermit crabs more often had spherical shells, whereas small crabs more often had conical shells. Our data do not support the hypothesis that resource limitation influences shell fit or shape in hermit crabs.

**Key words:** hermit crabs, shells, resource limitation, animal behavior

**INTRODUCTION**

Organisms are often limited by the availability of one or more resources. For example, a limiting resource might be a nutrient, such as nitrogen or phosphorous. If released from nutrient limitation, organisms that take advantage of the increased resource can grow faster. Well-adapted animals tend to respond to resource limitation by altering their behavior to maximize capture of their most limiting resource.

Some organisms experience a more discrete form of resource limitation. Hermit crabs, which reside in gastropod shells, are limited by shell availability. They remodel discarded gastropod shells by dissolving some of the inner structures, providing more room for the hermit crab body and decreasing the weight of the shell to improve maneuverability (Laidre, pers. comm.). They cannot survive for extended periods outside of their shells because their soft, unprotected abdomens are vulnerable to both predation and desiccation (De Vosjoli 2005). Therefore, the size of a hermit crab population is directly limited by the number of available shells.

Individual shells vary in size and shape because they come from different species and sizes of gastropod. The size and shape of shell that is physiologically optimal for any individual hermit crab might also vary, meaning there is selection on hermit crabs to find the best fitting shell. Shell size affects hermit crab growth because hermit crabs that grow too large for their shells are more vulnerable to other hermit crabs removing them from their shells. Hermit crabs search for a slightly larger shell to move into to facilitate further growth. Because it is energetically costly for hermit crabs to remodel shells, it is preferable for a hermit crab to obtain a previously remodeled shell. However, almost all remodeled shells are usually already occupied by another hermit crab, making remodeled shells a limiting resource for hermit crabs. This leads to shell swapping cascades, in which several hermit crabs of different shell sizes each move into a slightly larger shell that was moments earlier occupied by another hermit crab. Hermit crabs in larger populations have more combinations of shell size and shape available in these swapping cascades simply because there is a larger market. In addition to size, different shell shapes could differ in physical properties such as strength, durability, and maneuverability. Variance in physical properties suggests that there might be a preferable shell shape. If so, hermit crabs of all sizes would prefer shells that are both slightly larger than their current shell size and of a particular shape. Therefore, shell size and shape would be limiting to hermit crabs because not all hermit crabs could occupy their preferred size and shell shape.
At El Campanario Biological Station on the Osa Peninsula in Costa Rica, hermit crabs occupy two distinct shell shapes: conical and spherical. The physical properties of different shell shapes might make one shell shape preferable over others due to the advantages it confers, such as increased speed of locomotion. Organisms are known to alter their behavior to maximize acquisition of their most limiting resource. Therefore, if one shell shape is superior, hermit crabs should prefer that shell shape.

We studied hermit crabs from populations from two distinct beaches, one which had a much higher abundance of hermit crabs. Higher hermit crab abundance increases the number of potentially available shells and the variation in shell size and shape by providing access to a larger market. If finer differences in shell size and shape allow hermit crabs to fit their shells better, then the larger population of hermit crabs should demonstrate better shell fit.

At El Campanario, conical shells seemed more abundant among smaller shells, whereas spherical shells seemed more abundant among larger shells. If the frequency of conical and spherical shells differs between size classes, then there are different limitations of shell shape in different size classes. Preference for a superior shell shape and unequal distributions of shell shapes between size classes would lead to increased competition between hermit crabs.

METHODS

We collected hermit crabs from two beaches at El Campanario Biological Station on the Osa Peninsula. We recorded the body mass of each hermit crab, as well as the mass and shape of its shell. Spherical shells were characterized by having round edges and a general spherical structure and conical shells were characterized by having sharp edges and being wider along the lateral than the anterior/posterior axis (Figure 1). All statistical analyses were performed in JMP Pro 13 and Microsoft Excel.

Shell Fit

To determine whether the population size of hermit crabs affected the fit of the shells for individual hermit crabs, we surveyed two beaches, one with an observed higher abundance. We collected 40 crabs from the low abundance beach and 39 crabs from the high abundance beach and measured their total mass (including shell) and claw length as a proxy for body mass. We used the residuals from a regression of total mass on claw length as a measure of shell fit. We tested for patterns in shell fit with respect to population size and shell shape using a two-way ANOVA.

Speed

To test the potential benefits of one shell shape over the other, we performed a speed test. We presented large hermit crabs in conical (n=5) and spherical (n=9) shells, collected from the high abundance beach, with a 48-cm racecourse with a piece of raw coconut at the end as incentive. We timed how long it took each hermit crab to reach the end of the course, once it started walking. We used a linear regression to test for an effect of mass on speed. In the case that there is no effect, we would perform a t-test using speed as the dependent variable. In the case that there is an effect, we would perform a t-test using the residuals of the regression to determine whether shell type affected speed while accounting for the effects of mass.

Preference

To test whether hermit crabs preferred one shell shape over the other, hermit crabs were taken out of their shells and placed in a sandbox 5 cm away from one spherical and one conical shell. We recorded the shell shape the crab chose. We performed a chi-square test to determine if hermit crabs chose one shell shape more often than the other, given their original shell shape.
Frequency Distribution of Shells
To measure the frequency of spherical and conical shells for different hermit crab size classes, we walked a 60-m transect on the high abundance beach, once in the morning to count the shell shape distribution in small crabs (usually out in the morning) and once at night to count the shell shape distribution in large crabs (usually out at night). Crabs smaller than 11 g were classified as small, and those larger than 11 g as large. To test whether the shell shape was significantly different across size classes, we performed a chi-square analysis.

RESULTS
Shell Fit
There was a strong effect of claw length on total mass of hermit crabs (slope = 1.22 ± 0.05, $P < 0.001$, $r^2 = 0.87$, Figure 2). Shell fit was similar between the low and high abundance populations ($F_{1,78} = 1.02, P = 0.32$) and between shell shapes ($F_{1,78} = 2.49, P = 0.12$).

Speed
We measured speed in 14 hermit crabs. Of these, 9 had spherical shells and 5 had conical shells. Total mass did not affect speed (slope = $-0.83 ± 0.66, P = 0.25, r^2 = 0.18$). Hermit crab speed did not differ between shell shapes ($t_{7.8} = -0.73, P = 0.48$; Figure 3).

Preference
We presented 13 hermit crabs with a shell preference test. Of these, 7 originally had spherical shells and 6 had conical shells. Nine hermit crabs chose conical shells and 4 hermit crabs chose spherical shells. Hermit crabs did not show a significant preference between shell shapes, regardless of original shell shape (chi-square = 0.03, $P = 0.85$, df = 1; Table 1).

Table 1. Hermit crab shell shape preference, given original shell shape.

<table>
<thead>
<tr>
<th>Preferred</th>
<th>Spherical</th>
<th>Conical</th>
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<tbody>
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<td>Conical</td>
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<tr>
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<tr>
<td>Total:</td>
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</table>

Frequency Distribution of Shells
Conical shells were more abundant in small hermit crabs and spherical shells were relatively more abundant in large hermit crabs (chi-square = 45.2, $P < 0.001$, df = 1). We did not observe any empty hermit crab shells.

DISCUSSION
Our results did not support the hypothesis that a larger population, and therefore a greater availability of shells, would allow hermit crabs to have a better shell fit. This might be because hermit crabs biologically regulate their growth; therefore, they usually grow to fill their current shell and not beyond it. The fit of a hermit crab's shell, then, is determined by its biology and would be independent of the availability of shells in the population. We also found that shell shape had no effect on crab speed and that there was no significant difference in hermit crabs’ shell shape.
preference. Our sample size, however, might have been too small for a biologically meaningful difference to be statistically significant. Of the crabs we surveyed, there was a trend towards a preference for conical shells. Although shell shape did not affect speed, the slight preference towards conical shells that we observed might be due to other benefits we did not test. In the future, it would be interesting to further study whether crabs have a preference for spherical or conical shells, and if the preference is motivated by benefits in shell durability or strength.

We observed that small hermit crabs were significantly more likely to have conical shells, whereas large hermit crabs were more likely to have spherical shells. We might have observed this because the gastropods that produce spherical shells might grow larger than gastropods that produce conical shells. This phenomenon would directly affect the shape of the shells available for hermit crabs as they grow larger.

Although hermit crabs are limited by the abundance and size of shells, our results do not support the hypothesis that there is an optimal combination of shell size and shape. Hermit crabs seem to fit well in the shells they occupy and we observed neither preference nor a benefit to having a spherical or conical shell. If future studies find more conclusive support for a shell preference or that there are other benefits to a particular shell shape, then the difference in frequency that we observed suggests differences in shell limitation and competition between size classes.

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LITERATURE CITED
ANT TOUCH THIS: *Atta colombica* PHEROMONE TRAIL RECOVERY AFTER DISTURBANCE

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Faculty Editor: Hannah ter Hofstede

**Abstract:** Eusociality is a phenomenon that occurs primarily in hymenopteran insects, likely due to their haplodiploid sex determination system. For leaf-cutter ants (*Atta colombica*), pheromone trails are essential for traveling from the nest to trees for harvesting leaves. A disturbance or break in the pheromone trail can decrease the efficiency of the trail. Trail ant community, defined here as the distribution of small, large, and leaf-bearing ants, and obstacle width could alter the success of pheromone trail re-establishment. Additionally, abundance of ants on the trail may change the success of recovery. We studied one colony of *A. colombica* at El Campanario Biological Research Station in Costa Rica. We used two obstacle widths (25 mm and 50 mm) to create a disturbance in the pheromone trail and then measured trail recovery in relation to initial trail ant community and ant abundance. Our results show no difference between the percent recovery of trails across different ant class communities. However, higher abundance of all ant classes on the trail had a negative effect on percent recovery (number of ants to cross an obstacle compared to number that crossed prior to obstacle) and positive effect on absolute recovery (total number of ants to cross an obstacle). Increasing the width of the obstacle greatly reduced the success of re-establishing a pheromone trail across all ant class communities. Our findings have implications that ant fitness is related to colony survival and growth rather than individual survival.

**Key words:** *Atta colombica*, eusocial, leaf-cutter ants, pheromone trail

**INTRODUCTION**

Almost all organisms are inherently selfish, and will work to increase their own fitness even when living cooperatively. Hamilton’s rule (rB > C) offers an explanation of behavior that appears selfless: the product of an organism’s relatedness to another individual (r) multiplied by an action’s benefit (B) must be greater than its cost (C) in order for the organism to engage in that action for the benefit of another individual. The difference between normal organisms and eusocial insects is that sisters of eusocial insects are more closely related to each other than to their own offspring and so the relatedness coefficient of Hamilton’s equation is high between eusocial individuals. Therefore, the benefit of helping the group pass on these highly shared genes outweighs the cost to each individual of forgoing reproduction.

Leaf-cutter ants (*Atta colombica*) represent one of several insect taxa that have converged on eusociality. Leaf-cutter ants use pheromone trails to guide each other to important locations, such as to and from the nest and leaf harvesting sites. Without the pheromone trail, these ants have extreme difficulty finding their way back to the colony. A disturbance in the trail leaves that portion of the trail without pheromones, impeding the ants’ ability to collect food and return to the nest. Even minor disturbances like a fallen leaf or twig can temporarily obscure the pheromone trail, effectively halting traffic until the trail is re-established. To re-establish the trail, individual ants must risk walking into this informational “void” to get leaves back to the colony.

Leaf-cutter ants have a caste system in which ants of different body sizes perform different tasks (DeGroote et al. 2014). If specific types of ants are indeed better at different tasks, then trail ant community, defined as the frequency distribution of small, large, and leaf-bearing ants, might affect the successful re-establishment of the pheromone trail, defined as the complete recovery of the trail to the same number of individuals as was present before a disturbance. One possibility is that leaf-bearing ants might simply be more motivated to return to the nest because they have already invested energy in carrying their load from the tree (DeGroote et al. 2014). In this case, perhaps leaf-bearing ants will contribute the most to successful trail re-establishment. Another possibility is that smaller ants might have a role similar to maintenance workers, so they will be the most effective at re-establishment. Evison et al. (2007)
found that small ants deposit pheromones on the trail more often than large ants. A third possibility is that body size is the most important factor in crossing gaps, so ants with large body sizes will be more successful at breaching gaps simply by virtue of being longer.

To investigate how trail ant community, the number of ants in a trail, and the size of an obstacle might influence the success of ants at overcoming an obstacle, we measured two metrics of success: percent recovery (the proportion of ants successfully crossing an obstacle) and absolute recovery (the total number of ants successfully crossing an obstacle). Percent recovery addresses success on the scale of the trail whereas absolute recovery more accurately addresses success on the scale of the colony. Whichever class of ant contributes most to successful trail re-establishment, one would expect that the higher the number of ants of that class on a trail, the higher the percent and absolute recovery of the entire trail after introduction of an obstacle. Alternatively, more diverse groups of ants might be better problem-solvers, making trails not dominated by any one type of ant the most successful at re-establishment. If that were the case, one would expect that trails in which there is an equal ratio of distribution among ant classes to have the highest percent and absolute recoveries. Total ant abundance might also impact trail recovery, where areas of higher traffic are expected to be more successful by both metrics. The width of a disturbance might also impact the efficacy of pheromone trail re-establishment.

METHODS
From 31 January to 2 February 2017, we sampled a trail from one *A. colombica* colony at El Campanario Biological Research Station in Costa Rica. The trail was only active at night, so testing was conducted from 1800 to 2100. We selected 13 sites along the trail based on ease of access, and sites were approximately 3 m apart. We measured the distance from the nest to each sampling site (sites ranged from 4.3 m to 40.2 m from the nest). At each site, we recorded the time and then counted the total number of small, large, and leaf-bearing ants moving outbound and inbound past a specific point for one minute. Next, we placed our obstacle across the entire trail, either half a piece of duct tape (25 mm) or a full piece (50 mm) wide, on the ground. Obstacle length varied by site and tape was cut to ensure it crossed the whole trail. We waited for one minute and then conducted a second one-minute count of ants of each class that were able to cross the duct tape in either direction. Percent recovery of the pheromone trail was determined by dividing the total number of ants crossing the trail during the observation period following obstacle placement by the number of ants crossing the trail during the observation period prior to obstacle placement. The ant classes were determined by visual observation of large ants (larger than 5 mm), small ants (smaller than 5 mm), and leaf-bearing ants (any ant that was carrying plant material of any type). The leaf-bearing ants were not separated into size classes. To collect ant class size measurements, we collected ten or more ants from each class. These ants were taken back to the lab and we measured their length using calipers.

We performed a series of two-way ANOVAs to test the effect of abundance of each ant class and ant community evenness on percent trail recovery. Ant community evenness was defined as how similar the abundances of each of the three ant classes were to each other. It was quantified by conducting chi-squared tests on the sets of ant class abundances for each trail observation, with expected values calculated as perfect thirds of the total ant abundance. We then took the natural log of the chi-squared values for each observation to ensure normal distribution. We used a two-way ANOVA to test the impact of total abundance of ants and obstacle width on percent recovery. We tested for an effect of the total number of ants crossing at each site before the obstacle was placed on absolute recovery using a linear regression. We conducted a two-way ANOVA to test how ant class and obstacle width impacted percent recovery of the pheromone trail. All statistical analyses were conducted in JMP Pro 13 and Microsoft Excel.

RESULTS
Ants measured in the small class were 3.45 ± 0.12 mm long (n = 12), large ants were 6.4 ± 0.12 mm long (n = 11), and leaf-bearing ants were 7.05 ± 0.17 mm long (n = 10). Since leaf-bearing ants were not significantly different in size from the large ants, we grouped large and leaf-bearing ants...
together for analyses of the effect of size on percent recovery.

The number of leaf-bearing ants ($F_{2,22} = 9.70, P = 0.07$), number of large ants (with and without leaves, grouped by size for this analysis) ($F_{2,22} = 6.89, P = 0.66$), and evenness of trail community ($F_{2,22} = 7.62, P = 0.30$) all had no effect on percent recovery. The number of small ants had a negative effect on percent recovery ($F_{2,22} = 14.07, P = 0.006$). In our 26 observations of trail sections, there was no significant difference between the percent recovery of different ant classes ($F_{2,63} = 1.14, P = 0.33$; Fig. 1).

Total ant abundance (of all ant classes) had a negative effect on percent recovery ($F_{2,22} = 12.6 = 12.6, P = 0.01$; Fig. 2). As the total number of ants before the obstacle was placed increased, number of ants crossing after the obstacle was placed increased linearly (slope = 0.34 ± 0.08, $P < 0.001, r^2 = 0.44$; Fig. 2).

In the field, we observed that as distance from the nest increased, the number of ants decreased (slope = 4.01 ± 0.66, $P < 0.001, r^2 = 0.64$; Fig. 3). Mean percent recovery for all ant classes together for half width obstacle trials was 81.74% ± 7.56%, while mean percent recovery for full width obstacle trials was 26.36% ± 7.89%. While ant class does not impact recovery, full width obstacles decreased total ant abundance in terms of total number of ants crossing an obstacle (Fig. 4).

DISCUSSION

Small ants were the only class whose abundance had an effect on percent recovery. Higher numbers of small ants on the trail before disturbance resulted in less successful trail re-establishment in terms of percent recovery. This pattern held true for ant abundance in general: higher numbers of ants of all classes on a trail reduced the trail’s percent recovery. However, judging success by
absolute recovery suggests an opposite trend, wherein a greater number of ants on the trail before the obstacle was placed led to a greater total number of ants crossing the obstacle. If trail re-establishment success is measured by percent recovery, sparse trails appear more successful, but if the metric is absolute recovery, dense trails appear more successful. It is important to consider both metrics of recovery because each one is based on different implicit assumptions about what is most important to the colony’s fitness. The assumption associated with percent recovery is that efficient use of workers is what matters to the colony. The assumption associated with absolute recovery is that the colony benefits most from more ants returning to the nest.

If success is judged based on percentage recovery, one could conclude that ants are inept in large groups. The more ants on the trail at the time of a disturbance, the more the effect of that disturbance becomes magnified, just like a lane closure on a freeway. It is a surprising dynamic for eusocial insects (whose power comes from unity and strength of numbers) to be worse at accomplishing a task, in terms of percent trail recovery, en masse. As Garczynski and Vestergaard (2015) suggest, it is easy to assume that more ants means a larger group effort and thus higher recovery, but in reality space limitations cause crowding on the trail which further compounds the problem. However, in terms of absolute recovery dense trails still might be more beneficial to the colony because more ants get across the obstacle and reach the nest.

Eusocial insect colonies function on the premise that each individual is disposable, so selection may not be acting to improve individual ability to overcome obstacles on the pheromone trail. If selection were to act on individuals to make each one better at overcoming trail obstacles, then each individual would be more expensive to create (more reproductive investment in a higher-quality individual), thus undermining its disposability. This may be why even individuals from a successful colony are not successful on their own. It might actually be in each ant’s best interests to ignore the costs of high numbers of ants getting stuck behind obstacles in high-traffic situations. In spite of the fact that traffic flow recovered to a lower percentage of its original level on dense trails than sparse trails and thus was less efficient in terms of worker use, the total number of ants that made it across the obstacle was still higher on dense trails. The main goal for all ants is to get leaves back to the nest, and so it might prioritize this goal over the efficient use of each individual. Under this logic,
high traffic flow trails might be less maladaptive than they initially appear. The “waste” of individual ants that results from lower proportional recovery with higher initial abundance might not matter to each individual's fitness as much as getting more resources to the nest.

It is not unexpected that more ants are on the trail closer to the nest, because ants of all classes exit the nest early in the night to forage, and some ants are already returning to the nest with leaf fragments. If ants have higher percent recovery when there are fewer individuals present, and there are more individuals near the nest, then as a group they are worse at overcoming obstacles closer to their nest. However, in terms of absolute recovery, having more ants closer to the nest will result in more total ants crossing an obstacle. While the trail may lose a higher percentage of individuals near the nest, having a higher total number of ants cross the obstacle will ultimately be most beneficial to the colony as a whole.

For both absolute and percent recovery, a wider obstacle decreased the success of re-establishment relative to the narrower obstacle. A larger obstacle increases the distance of the new pheromone trail that must be established. Regardless of ant size or energy invested in bringing a leaf-load to the nest, a larger distance will require more effort to recover a trail and, therefore, will take longer to overcome. If small ants are more likely to re-establish a pheromone trail because they lay down pheromones more frequently, then a wider obstacle, even by 25 mm, will greatly impact recovery. A 25 mm increase in obstacle width would be smaller in comparison to a big ant and therefore, would be likely to have less of an influence on percent recovery. However, we found no evidence that any class of ant was better at crossing pheromone gaps than the others.

The finding that each ant class is equally inept at trail re-establishment, suggests that colony success does not depend on the success of one ant class.

Despite the incredible feats accomplished by leaf-cutter ants on the colony level, as individuals they are largely inefficient at overcoming obstacles. The high relatedness between sister workers means that their fitness is increased by assisting the growth of the colony rather than reproducing themselves, which has the interesting effect of reducing efficiency due to individuals being disposable. Our findings have implications that ant fitness is related to colony survival and growth rather than individual survival. Thus, eusocial insects may make good subjects for future studies of relative versus absolute fitness.

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LITERATURE CITED
La Selva
COLORATION IN THE HONDURAN WHITE BAT (ECTOPHYLLA ALBA): IT’S NOT BLACK AND WHITE

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Abstract: Organisms often resemble the environments in which they live for more effective camouflage from both predators and prey. In addition, body coloration can impact thermoregulation in animals since dark colored objects absorb more radiant energy than light ones. The majority of bat species are darkly colored and resemble the cavernous backgrounds on which they roost. However, the Honduran white bat (Ectophylla alba) is white and roosts in tents constructed from Heliconia leaves. We investigated the potential effects of white fur color on temperature and visual contrast of the Honduran white bat. We examined the effects of color on the temperature inside modified Heliconia leaves by measuring the temperatures of a group of roosting Honduran white bats in a natural tent roost and of white and black body objects in human-modified Heliconia tent structures over the course of a day. We experimentally examined the effects of color on visual contrast by comparing contrast values of black and white body objects photographed from various positions in a leaf tent. Honduran white bat temperature remained relatively constant throughout the day. Moreover, white objects were significantly cooler than dark objects, indicating that color affects temperature within the leaf structure. In cooler contexts, this might not be beneficial. White coloration also significantly reduced contrast when viewed from below, suggesting that white color might be beneficial for evading ground-dwelling predators. When viewed from above or from the same side as the source of light, the leaf obstructed the object entirely; when viewed from the side opposite from the source of light, a clear shadow was produced but was not significantly different for white versus black objects. These findings suggest that white coloration might negatively impact the Honduran white bat’s thermoregulatory energy expenditure in some cases yet improve its ability to conceal itself from predators while roosting. Thus, coloration in animals can result in tradeoffs and might be driven by the context in which animals are most vulnerable.

Key words: camouflage, coloration, thermoregulation

INTRODUCTION

There is often a resemblance between organisms and the substrates on which they live. As Alfred Russel Wallace remarked, “Arctic animals are white, desert animals are sand-coloured, dwellers among leaves and grass are green, nocturnal animals are dusky. These colours… are seldom reversed” (2016). This pattern provides the organism with a substantial fitness advantage by concealing it from both predators and prey. It follows that dark fur color is characteristic of the majority of bat species; their dark coloration might help them to go unnoticed when they roost in dark crevices and caverns. However, the Honduran white bat (Ectophylla alba) is unusual in that it roosts in modified Heliconia leaves and is conspicuously lacking in fur pigmentation (Timm and Mortimer 1976, Rodriguez-Herrera et al. 2006).

The Honduran white bat is native to Central America and is one of twenty-two bat species that roost in tented leaves (Rodriguez-Herrera et al. 2006). This bat alters Heliconia leaves by cutting the side veins extending from the midrib, causing the sides of the leaves to fold down (Timm and Mortimer 1976). This tent-like shelter can support a group of 1-6 individuals (Timm and Mortimer 1976). Less than four percent of bat species roost in leaf-structures and only two species have been documented modifying leaves to create roosting sites (Rodriguez-Herrera et al. 2006). Moreover, one other bat species, in which white coloration evolved independently (Dicliduris scutatus), is known to similarly roost in leaves (Sodrê and Uida 2006). This convergent evolution suggests white fur might be beneficial for bats roosting in leaves. Due to the tent-making strategy, Honduran white bats might experience different visual and thermal substrates than do non-tent-roosting bat species. This could put the Honduran white bat under different selective pressures than those experienced by darker, non-tent-making bats.
We examined the potential effects of white fur color on temperature and visual contrast in *E. alba*. White fur might have different heat absorption properties than dark fur for bats roosting in modified Heliconia leaves. For example, white fur might reflect more radiant energy. If this is the case, small white body objects in modified Heliconia leaves would consistently maintain lower temperatures than small black body objects in modified Heliconia leaves. Alternatively, white body color might not affect the heat-absorption capabilities of a small object in a tented Heliconia leaf. By preventing direct sunlight, the shade provided by the leaf could eliminate the relationship between temperature and color. White fur color might also impact the ability of bats to blend in with their environment. The color contrast between a black body object and a modified Heliconia leaf might be greater than the contrast between a white body object and the same leaf. Alternatively, body color might not affect the contrast of an object within a modified Heliconia leaf.

**METHODS**

**Temperatures of objects and leaves**

We identified a group of 4 *E. alba* bats in a Heliconia leaf tent in La Selva National Park. We recorded the temperature of the roosting bats using a thermal gun (Raytek) every half-hour between 08:25 and 16:50 on 11 February 2017. We also recorded the same temperature measurements for the part of the leaf adjacent to the bats. We modified four large Heliconia leaves within 0.25 km of the roosting bats to form a roosting structure resembling the tents made by the *E. alba* bats. We made perforations similar to ones made by bats by cutting a line of square holes (5 mm by 5 mm) about 1.5 cm apart on each side of the midrib of each Heliconia leaf and folding the leaves along these perforations to mimic the structure of the roosting tent. On the underside of each leaf, we secured two cotton balls with diameters of about 2.5 cm in the middle of the tent using fishing line. One cotton ball was colored black (black body object) and the other was colored white (white body object) with tempera paint. We randomized the orientation of the colored cotton balls under each leaf to minimize effects of cotton ball position on temperature. We recorded the temperature of each cotton ball under its respective leaf every half-hour using a thermal gun.

We performed a t-test to determine the effect of cotton ball color on cotton ball temperature. We performed a one-way ANOVA to test for an effect of object (bat, white cotton ball, or black cotton ball) on the difference between object temperature and leaf temperature. We performed linear regressions to examine the effect of leaf temperature on the temperatures of the bats, black body objects, and white body objects. We examined the interaction of leaf temperature and cotton ball color to test if the effect of leaf temperature on cotton ball temperature was significantly different between black and white cotton balls.

**Visual contrasts of objects in leaves**

On 11 February 2017 at 16:30, we collected and modified a large Heliconia leaf to form a tent structure and positioned the tent so that dusk sunlight shone on the side of the tent opposite the camera. We then positioned a black cotton ball at the base, middle, or tip of the tent and took one photograph at each marked location. We repositioned the camera to be on the same side of the leaf as the sun and took photographs of the black cotton ball positioned in the same locations (base, middle, tip). We replicated this procedure with a white cotton ball. We converted the photos to grayscale in Adobe Photoshop and quantified brightness using Hue-Saturation-Brightness values. We calculated contrast values by taking the difference in brightness units between a region of the leaf tent that contained a cotton ball and an adjacent region of the leaf tent that did not. We repeated the same photography and contrast analysis using midday sunlight (11:00) from above.
the leaf tent. We took photographs by directing the camera toward the cotton balls from above and below the tent.

For the trials with dusk sunlight, we performed a two-way ANOVA to determine the effects of cotton ball color, light source position, and the interaction of cotton ball color and light source position on cotton ball contrast. For the trials with midday sunlight, we performed a two-way ANOVA to determine the effects of cotton ball color, camera position (above or below leaf tent), and the interaction of cotton ball color and light source position on cotton ball contrast.

RESULTS

Temperatures of objects and leaves
Bat temperature (mean = 29.79 ± 0.24 °C) remained relatively constant throughout the day (Fig. 1A). The mean temperature of the white cotton balls (24.26 ± 0.10 °C) was significantly lower than that of the black cotton balls (24.92 ± 0.13 °C) over the course of the day ($t_{101.87} = -3.90$, $P < 0.001$, Fig. 1B). There was a significant effect of object type on the difference between object temperature and leaf temperature ($F_{2,125} = 251.54$, $P < 0.001$). A post-hoc Tukey HSD revealed that bats were warmest (mean = 4.27 ± 0.28°C), followed by black cotton balls (mean = -0.31 ± 0.15°C) and white cotton balls (mean = -0.96 ± 0.15°C). Leaf temperature did not have a significant effect on bat temperature (slope = 0.30 ± 0.19, $P = 0.14$, $r^2 = 0.15$, Fig. 2). There was a significant interaction between cotton ball color and leaf temperature on cotton ball temperature ($F_{1,108} = 6.87$, $P = 0.01$; Fig. 2).
**Visual contrasts of objects in leaves**

When the light source shone on the side of the leaf tent, the contrast was significantly higher when the camera was on the opposite side than on the same side of the light source because a shadow was produced ($F_{1,8} = 63.82, P < 0.001$; Fig. 3). In this situation, the contrast is unrelated to cotton ball color ($F_{1,8} = 2.35, P = 0.16$). There was also no significant effect of the interaction between the cotton ball color and light source position ($F_{1,8} = 0.19, P = 0.67$). When the light source shone from above the leaf tent, there was a significant interaction between camera location and cotton ball color on contrast ($F_{1,8} = 19.59, P = 0.002$; Fig. 4). A post-hoc Tukey HSD test revealed that the contrast (difference in brightness values) was highest for black cotton balls when the camera was below the leaf (61 brightness units), least for black cotton balls when the camera was above the leaf (1.33 brightness units), and intermediate for white cotton balls when the camera was below the leaf (1.67 brightness units) and white cotton balls when the camera was above the leaf (2.67 brightness units).

**DISCUSSION**

Bat temperature while roosting remained relatively constant despite fluctuation in leaf temperature. This indicates these bats maintain a constant internal temperature while roosting. The temperature of white body objects under leaf tents did not increase as much with increasing leaf temperature as black body objects, suggesting that white bats should expend less energy to stay cool and more energy to stay warm than do dark bats. It is possible that these thermal differences would have physiological consequences for *E. alba.*

During our observation period, staying warm might have been favorable. Rodríguez-Herrera et al. (2006) found that *E. alba* metabolic rate is negatively correlated with ambient temperature until it reaches 30°C or higher, at which point metabolic rate stays constant. Leaf temperatures did not reach 30°C at any point during our observations, so it is plausible that the bats were expending energy to maintain their body temperature while roosting. Daytime temperatures near the station only reached 30°C a few days between March and May of the past two years (2015-16) (www.wunderground.com). The tenting structures have been shown to collect heat, so it is likely that temperatures inside modified leaves.
approach 30°C more frequently than daily temperature data might suggest (Rodríguez-Herrera et al. 2006). In addition, the geographic range of *E. alba* extends south into western Panama, where average daily temperatures might be greater. However, the geographic range of these bats also extends northward into Eastern Honduras and we do not have sufficient evidence indicating that internal modified leaf temperatures regularly surpass 30°C (Ellis, E. 1999). This suggests that white fur does not usually offer a thermoregulatory advantage for these bats and instead might increase thermoregulatory costs.

Our results indicate that, when viewed through the leaf, fur color does not affect the visibility of *E. alba* to predators. The leaf conceals both white and black bats when viewed from the side or above, except when backlit. Although both white and black body objects were visible through the leaf when backlit, such lighting would occur at most twice a day (i.e., during sunrise and sunset) and would be minimized due to low levels of direct, low-angle sunlight near the forest floor. Thus, it is unlikely that the sunlight reaching the leaves would be sufficient for producing clear shadows of the bats’ bodies. These results suggest that *E. alba*’s tent-building strategy is adequate for concealing it from aerial or large terrestrial predators.

However, white fur seems to allow tent-roosting *E. alba* individuals to camouflage themselves more effectively from observers underneath the leaf, making bats less conspicuous to ground-dwelling predators such as snakes or raccoons. When viewed from below, white fur contrasts less against a green leaf than does black fur. It is also likely that white fur would be better able to reflect green light that passes through the leaf. Our contrast analysis required a conversion to grayscale and therefore cannot parse out whether or not reflected green light contributed to the observed results. Subsequent analyses accounting for color could address the importance of this mechanism for *E. alba*.

It appears that the selective pressures associated with thermoregulation and predator avoidance might act on color in opposing directions for *E. alba*. Our results suggest white fur might be energetically counter-productive for thermoregulation but beneficial for camouflage against predators underneath leaf tents. Because white is an extreme on the coloration spectrum, there are likely additional drivers that have selected for white fur over evolutionary time. Future studies should investigate the effect of white coloration on other factors, such as identification of conspecifics for mating, which might contribute additional selection pressure for this coloration.

Although *E. alba* is not adapted for camouflage in the context in which it is most active (i.e., it is not darkly colored for camouflage in the night) our results suggest that *E. alba* is instead adapted to camouflage in the context in which it is most vulnerable. *E. alba*’s white coloration aids it in resembling the leaf in which it roosts. Because these bats roost in leaf tents during the day close to the ground, this context is the one in which they are most at risk of predation and might benefit most from camouflage. Thus, *E. alba* is not a counterexample to the generalization that species will evolve to match their environments, but instead is a powerful example of it. The appearance of species that experience variable backgrounds is likely to be most strongly affected by the background in which stakes are highest.

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AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE Cited

APPENDIX
A competing hypothesis consistent with Wallace’s assertion that species evolve to resemble their environments has been proposed to explain the presence of the Honduran white bat in Costa Rica. It has been suggested that the Honduran white bat evolved in the circumpolar region. The northern limits of many bat species are not well documented, but there is evidence for breeding populations of at least one bat species (Eptesicus nilssonii) above the Arctic Circle (Rydell et al. 1994). The plausibility of this hypothesis is therefore deserving of consideration.

Under the Arctic Bat Hypothesis, the white fur characteristic of the Honduran white bat is adaptive for camouflage and predator avoidance in the tundra. This characteristic has convergently evolved in many Arctic species, including the formidable polar bear, the Arctic hare, and the Arctic fox. Thus, it is plausible that the white fur once served an adaptive purpose for bats. Unfortunately, cold winters are harsh on weary bones and caribou do not make good company, so in an unprecedented mass-migration event the species sought sunnier skies in the south. After a brief stay in Florida, the bats found there was not much to do other than drink and soak up rays at the beach. Bored, the entire population of Ectophylla alba sought out a more geographically interesting setting and migrated to Central America, settling in eastern Honduras through western Panama. Although nothing of the scale required for the Honduran white bat has been previously recorded, it is known that some bat species partake in long-distance, large-scale seasonal migrations. Thus, it is likely that this migration began as a seasonal migration from which the bats never returned.

However, clear paleontological support for the Arctic Bat Hypothesis is sparse. It is thought that a small delegation of European ecotourism hotel owners in Central America realized that such a tale would encourage research and tourism in the northern latitudes by stimulating interest in the aforementioned mass migration. To reduce potential competition and maintain tropical dominance in the tourism trade, this delegation might have removed all chiropteran remains or ecological evidence of the Ectophylla alba migration from the Canadian Arctic. They might still be working tirelessly to conceal any hint of the bat’s arctic past discovered in the fossil record. Or so the hypothesis goes.
APOSEMATISM AND VIABILITY FITNESS IN *OOPHAGA PUMILIO*

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**Abstract:** In many species, females choose male partners by assessing their viability fitness. In aposematic species such as *Oophaga pumilio*, the Strawberry poison dart frog, conspicuous coloration leads to higher viability fitness due to lower predation risk. Conspicuousness might be correlated with other traits affecting viability fitness such as jump distance and body size. At La Selva Biological Station in Costa Rica, *O. pumilio* are characterized by red backs and blue legs. If conspicuousness is a good indicator of overall viability fitness, redder males should also exhibit other indicators of fitness such as larger body size or better jumping ability. If redness correlates with other fitness proxies, females might prefer redder males. Therefore, male frogs might be redder than female frogs due to sexual selection. We analyzed the correlations between redness, jump distance, and body size in *O. pumilio*. We found no correlation among these proxies of viability fitness. However, females were significantly redder than males. This might be because females are more toxic due to differences in life history between sexes, or because sexual selection is acting as a pressure on females and not males. Our results suggest that color phenotype in *O. pumilio* might be a product of multiple selection pressures.

**Key words:** Blue-jeans frog, sexual selection, Strawberry poison dart frog, toxicity

**INTRODUCTION**

In all species, different evolutionary forces such as predation pressure and sexual selection interact to shape the appearance of individuals. In many species, females assess potential mates for cues or signals related to viability fitness (ability to survive) to give their offspring the best possible traits. There are many correlates of viability fitness, including color, body size, and performance, which can often be signs of better health or genes. In species with anti-predation defenses such as toxicity, conspicuous coloration serves as a warning to potential predators. Conspicuous coloration allows aposematic species to better avoid predation by increasing visibility to predators. Females might use aposematic coloration as a cue for viability fitness in mate choice.

Strawberry poison dart frogs (*Oophaga pumilio*) become toxic by eating alkaloid-containing arthropods and signal their toxicity to predators through aposematic coloration (Maan and Cummings 2012). Tadpoles become toxic by eating unfertilized eggs that the mother lays for them in bromeliads. *O. pumilio* are strikingly polymorphic across different populations of frogs. Different color morphs also differ in toxicity, with more conspicuous colors corresponding to increased toxicity (Maan and Cummings 2012).

Female *O. pumilio* use visual cues in mate choice, and differences in color are the most likely visual cue (Summers et al. 1999). However, it is not known whether females prefer males with more conspicuous coloration. Color polymorphism between populations of *O. pumilio* might be due to sexual selection in which females select for conspicuous coloration in males (Reynolds and Fitzpatrick 2007).

Conspicuous coloration is not the only indicator of viability fitness in *O. pumilio*. Jumping distance might reflect a frog’s strength or ability to escape predation, and body size might reflect foraging success. Male *O. pumilio* hold territories, and courtship involves a lengthy period of time in which males and females hop through the forest together (Summers et al. 1999), so females have ample time to assess the jumping performance of males. Although there is no biological reason for conspicuousness to cause frogs to be larger or jump farther, these different proxies of viability fitness could be correlated. Conspicuousness might be an indicator of other proxies of viability fitness because it might be increased by better foraging ability. Alternatively, conspicuousness might not be an indicator of other proxies of viability fitness because it might not be fully heritable and instead depend largely on diet. If conspicuousness is a good indicator of overall
viability fitness, females might prefer males with more conspicuous coloring. Additionally, if females are the choosier sex, there might be sexual selection pressure within the population favoring males with more conspicuous coloring.

At La Selva Biological Station in Costa Rica, *O. pumilio* are characterized by red backs and blue legs. For this particular morph, we used “redness” as a proxy for conspicuousness in coloration. If aposematic coloration is a good indicator of overall viability fitness, redder frogs will both be larger and jump farther than less red frogs. If this is true, we could expect females to prefer redder males, because more conspicuous color would indicate higher fitness. Therefore, if females prefer redder males, and females are the limiting sex, males should on average be redder than females.

**METHODS**

We collected data on *O. pumilio* at La Selva Biological Station in Costa Rica. We caught frogs by hand along trails in the reserve and brought them to the laboratory. We recorded the sex of each frog surveyed. Frog sex was determined by the width of the abdomen and the arch of the back; female frogs have a higher back arch and wider abdomen than male frogs. We also measured frog mass in grams.

To quantify the conspicuousness of the *O. pumilio* surveyed, we took pictures of all the frogs against a white background using an iPhone 5 and a light source positioned ten inches above the white background. We selected an area of the frog’s back (approximately 100 by 60 square pixels) and analyzed the saturation of the red hue of each pixel in the image using ImageJ. The selected area was limited to parts of the frog’s back without highlights or shadows. The saturation of red value is a measurement that ranges from 0 to 255. The mean red value is calculated by averaging the red value of all the pixels in the selected area of the image. The mean red value of each frog was used as the metric from which we determined “redness”.

To test for initial jump distance, we sprayed each frog with water and placed it on white paper towels, then measured the distance between the watermarks left at their starting point and first landing point after jumping (Goedert D, pers. comm.).

To test whether redness was related to greater attractiveness in male poison dart frogs, we placed a female frog with two male frogs of differing redness (determined by the mean red values from ImageJ) in a 30 by 15 cm container and observed which male the female jumped towards first. The observations were done in a three-minute period. We performed three trials, each using different female and male frogs.

We tested for correlations between body mass, initial jump distance and redness to determine whether redness was correlated with other fitness proxies. To test if redness differed between sexes, we performed a t-test. All statistical analyses were performed using JMP Pro v. 13.

**RESULTS**

We surveyed 27 *O. pumilio* with mean (± 1SD) red saturation values ranging from 88.41 ± 16.12 to 170.04 ± 17.02. Redness was not correlated to jump length (*r* = -0.26, *P* = 0.18, df = 25) or body mass (*r* = -0.27, *P* = 0.32, df = 25). Jump length and body mass were also not correlated (*r* = -0.21, *P* = 0.31, df = 25). Preference tests were inconclusive. Female frogs did not approach or interact with male frogs during the three preference trials. Both females and males spent time attempting to escape the container by climbing up the container walls, or jumping in seemingly random directions around the container.

![Figure 1. Female frogs were significantly redder than male frogs.](image-url)
One male vocalized 19 times during one of the trials, but the rest of the frogs were silent during the preference tests. Females were significantly redder than males ($t = 2.36, P = 0.03, df = 25$; Fig. 1). Mean (± 1SD) red saturation values for female frogs ranged from $126.8 ± 11.4$ to $169.0 ± 12.6$ while mean red saturation values for male frogs ranged from $88.41 ± 16.12$ to $170.04 ± 17.02$.

DISCUSSION
We found no correlations among color, jump distance, and mass. Redness, in this case, is not a representation of the two other forms of fitness. Therefore, level of redness is not an indicator of overall genetic fitness. Our results suggest that female *O. pumilio* might benefit from choosing mates based on a metric other than coloration. As a signal, coloration cannot convey information to females about male quality in the traits of body mass and jump length. However, redder coloration in and of itself provides a fitness boost by helping frogs avoid predation. If females were to choose mates based on color, they might still be able to pass on the high quality trait of conspicuous coloration to their offspring. However, they would have no way of selecting for other fitness traits based on this visual cue. In their mate selection, *O. pumilio* might have to make trade-offs between fitness traits because one individual does not have every ideal trait. It is possible that body size and jump distance are not good proxies for overall viability fitness. Future studies could test for more potential proxies for viability and female preference.

While we did not find a correlation between color and our chosen metrics of viability fitness, we did find a relationship between coloration and sex. In most species with dimorphic coloration, the males are generally more colorful to attract females. However, in these *O. pumilio* we found the opposite: females tended to have a redder coloration than males of the same species. This suggests that we might need to reassess a fundamental assumption about their life history: perhaps in *O. pumilio* males are the choosier sex. Future studies should assess the mating system of this species to see how mate selection occurs, if conspicuousness is a factor in mate preference, and which sex is exerting sexual selection pressure on the color of the other.

Theories on how sexual selection shapes coloration are predicated on the assumption that coloration is heritable. In these frogs, redness might not be a fully heritable trait and likely depends partially on diet. At the population level, *O. pumilio* that eat more toxic prey are able to synthesize higher toxicity for themselves and so can maintain more conspicuous aposematic coloration (Maan and Cummings 2012). Given this pattern, an alternate hypothesis explaining the difference in coloration between males and females is that females are more toxic than males. Females in this genus lay unfertilized eggs for their offspring to eat, passing on some of their toxicity. Females might need to sequester more toxins in their bodies to be able to donate some to their young while still maintaining their own toxicity. This difference in parental investment could explain the difference in coloration between males and females. Indeed, Stynoski et al. (2014) found that female *O. pumilio* at La Selva contained significantly more alkaloid toxins than their male counterparts, which they concluded might be because females need to sequester enough toxins to supply both themselves and their offspring. Thus, the sexual dimorphism in *O. pumilio* might be a narrow example of a broader biological pattern across all taxa: the more toxic the species, the more conspicuous their aposematic coloring.

Clearly, females invest a significant amount of time and energy in each round of reproduction. However, male *O. pumilio* also invest in reproduction: they must maintain a territory with enough bromeliads for each tadpole to have its own, and they engage in parental care by guarding and moistening the eggs before the tadpoles hatch. The amount of reproductive investment required ensures that females are reproductively unavailable for some periods of time, but male investment could still equal or potentially surpass it. If there are more females reproductively available in this population, males might be the choosier sex. Males might be exerting sexual selection pressure on females to have more conspicuous coloration because conspicuousness is an indicator for toxicity. Because females impart toxicity to their offspring by feeding them unfertilized eggs, males that mate with more toxic females might ensure that their offspring are more likely to survive.
Sexual selection occurs in situations where there are fewer reproductively available individuals of one sex than the other, so the sex that invests more is typically more selective. These sexual selection pressures can then influence the phenotypic traits in a population. Our findings suggest that multiple proxies of fitness are not correlated, so the more selective sex might make trade-offs among favorable traits during mate selection. Natural selection continuously acts on the phenotypes generated by these choices. Thus, multiple selection pressures interact with each other in complex ways to define an individual organism’s phenotype.

LITERATURE CITED
Thermoregulation, or the regulation of internal body temperature, is a crucial aspect of physiological performance for many species. Most animals, including reptiles, fish, and most invertebrates, are poikilotherms: they allow, or can tolerate, large variations in body temperature in response to environmental conditions. On the other hand, birds and mammals are homeotherms; they maintain a relatively stable body temperature, regardless of fluctuations in external environmental temperature. Both thermoregulation strategies have significant consequences for metabolic rate and behavior of organisms in different environments. Since their metabolic kinetics vary with external temperature, poikilotherms generally exhibit lower metabolic rates, and thus lower activity levels, in colder environments. Conversely, at higher temperatures, poikilotherms experience an increase in metabolic rate and overall activity. Most homeotherms are endothermic, generating their own body heat via metabolic processes to maintain a constant internal temperature in colder environments. However, homeotherms can also conserve the energy expended for heat generation at warmer temperatures.

Although they are mammals, which are generally homeotherms, two-toed sloths (*Choloepus hoffmanni*) have been classified as facultative poikilotherms, meaning that they exhibit characteristics of both thermoregulation strategies (Cliffe et al. 2015). Studies in the early 1900s demonstrated that the internal temperatures of sloths (both two-toed and three-toed) vary with both ambient air temperature and exposure to sunlight (Kredel 1928, Britton 1938). A recent study of *C. hoffmanni* found that breathing rate increases linearly with temperature, supporting the idea that the metabolism of *C. hoffmanni* increases with temperature (Costello and Rieb 2012). Other studies have shown that the activities of sloths can be affected by the slight variation in their internal temperatures as a result of ambient temperature changes. For example, a study of the brown-throated sloth (*Bradypus variegatus*) found that increased external temperature resulted in increased food consumption (Cliffe et al 2015). There is clear evidence that ambient temperature can alter not only sloth metabolism, but also the resulting behaviors of the organism in ways that can impact its survival.

As facultative poikilotherms, the internal temperature of *C. hoffmanni* should rise with the external temperature, resulting in increased activity levels, as well as an increase in time devoted to active behaviors such as climbing and foraging. In this study, we investigate the relationship between ambient temperature and behavior in *C. hoffmanni*, measured in terms of activity duration throughout the day.
METHODS
Field Observations
We conducted research from 10 to 12 February 2017 along Sendero Arriera Zampopa and on the Stone Bridge at La Selva Biological Research Station in Puerto Viejo de Sarapiquí, Costa Rica. Using a focal individual study with 60-minute observation periods, we observed the behavior of four *C. hoffmanni* individuals. Observation took place from 0800 – 1130 (morning), 1300 – 1730 (afternoon), and 2000 – 2100 (night). We recorded the duration of each activity that took place during each observation period. The distance between observers and individuals varied from 2m to 20m, and we viewed individuals using Bushnell and Vortex-brand binoculars (8x42) when necessary. Given frequent human activity on the trails and bridges of La Selva, we assumed that these *C. hoffmanni* individuals are habituated to human presence; therefore, we did not use blinds during our observation periods. Due to the difficulty of sexing *C. hoffmanni* individuals, we did not record the sex of study subjects.

We classified activities into seven categories: hanging, hugging, moving, climbing, foraging, stretching, and scratching. Hanging and hugging were the two resting postures of the sloths; individuals either hung from a branch while resting, or propped their bodies directly against the branch in a hugging position (Figure 1). The individuals spent a notable amount of time scratching themselves, so this activity was given its own category. We labeled any general movement of the individual’s body that did not result in a change in location in the tree as moving. On the other hand, if the individual moved its entire body spatially in any direction, we labeled its activity as climbing, and if the individual was eating, drinking, or climbing between patches of leaves, we labeled this activity as foraging.

We also recorded weather, noting precipitation, cloud cover, sunlight, and whether the individual was in the shade or sun. Temperature was recorded using HOBO loggers placed in the shade in the forest close to study individuals, and recorded temperatures were matched to the times of observation.

Statistical Analyses
We used a one-way ANOVA to determine the effect of time of day (morning, afternoon, and night) on the proportion of time individuals spent moving during an observation period. We then used one-way ANOVAs to test the effect of weather (cloudy or sunny) on the proportion of active time and whether being in the shade or in sunlight impacted the proportion of active time of an individual. We also used a one-way ANOVA to compare the relative amount of time devoted to each activity across sloths. We conducted a logistic regression looking at the effect of ambient temperature on whether an individual moved in a ten-minute period. This regression was repeated separately for three of the individuals. Finally, we conducted a logistic regression to test the effect of ambient temperature on *C. hoffmanni* activity, the difference between individuals, and whether there was an interaction between individual *C. hoffmanni* and temperature. All statistical analyses
were conducted in JMP Pro 13 and Microsoft Excel.

RESULTS
In twenty-four 60-minute observation periods, we recorded five hours of movement for the four individuals. Climbing was the most common movement activity (total duration: 1:35) and hugging was the most common non-movement activity (total duration: 13:45; Figure 2).

We recorded the ambient temperature at one-minute intervals for a 24-hour period, and at hour intervals for a 72-hour period, and found that the temperature ranged from 19 ºC (measured around 2 am) to 30 ºC (measured around noon; Figure 3).

When looking at the relationship between temperature and activity, we excluded one individual due to a lack of reliable temperature data; the only HOBO set up during that observation was recording at hourly intervals. We also discarded the first round of observations for Sloth 1 for the same reason.

We found no relationship between time of day and the proportion of time C. hoffmanni devoted to activity ($F_{2,22} = 1.010, P = 0.381$). We also found no relationship between cloud cover and the proportion of active time ($F_{2,22} = 1.215, P = 0.316$). There was no relationship between the proportion of active time and whether the observed individual was in shade or sunlight ($F_{2,22} = 1.691, P = 0.207$). However, there was a significant difference overall in the amount of total time C. hoffmanni devoted to each activity ($F_{8,71} = 21.815, P <0.001$; Figure 2).

We found no relationship between temperature and activity when data was pooled for all three individuals (chi-square = 0.20, $P = 0.65$). However, at least one of the individuals behaved significantly differently than the others, shown by the results of a logistic regression looking at the effect of individual, temperature, and individual*temperature (chi-square = 16.55, $P = 0.005$). The effect of individual sloth was effectively significant (chi-square = 5.93, $P = 0.052$), and there was almost a significant interaction between individual and temperature (chi-square = 5.46, $P = 0.065$). We then investigated each subject individually, as we did not have the statistical power to account for large variation between study subjects due to our small sample size.

Sloth 1
Sloth 1 stayed within a 50m-area about 200m from the biological station along Sendero Arriera Zampopa. Across seven hours of observation ($N = 42$ ten-minute time intervals), temperature did not have an effect on activity level in Sloth 1 (chi-square = 0.03, $P = 0.86$; Figure 4). However, before beginning our recording period, on the morning of 10 February, we observed Sloth 1 climb with surprising speed from its original tree to a tree over 20m farther into the forest, traversing at least seven trees along its way. From
the beginning of our study period that afternoon to
the end of the study period, Sloth 1 produced
almost no activity and slept during most of the
observed time.

**Sloth 2**

Sloth 2 stayed within a 30m-range of several trees
along both sides of the eastern end of Stone
Bridge. Similar to Sloth 1, across eight hours of
observation (N = 48 ten-minute time intervals),
temperature did not have an effect on activity level
in Sloth 2 (chi-square = 0.90, P = 0.34; Figure 4).
While Sloth 2 was not very active during the day,
its location changed notably overnight.

**Sloth 3**

Sloth 3 stayed within a 20m-range of several trees
to one side of the eastern end of Stone Bridge,
overlapping in range with Sloth 2. However,
during observation, Sloths 2 and 3 were always
found in different trees. Unlike Sloths 1 and 2,
across 9.66 hours (N = 40 ten-minute time
intervals), there was a significant relationship
between temperature and activity level in Sloth 3
(chi-square = 4.90, P = 0.03; Figure 4). For Sloth
3, as temperature increased, activity decreased.
Sloth 3 was more active than the other two sloths
during the day, climbing and foraging frequently.
Furthermore, Sloth 3 was the only individual
observed climbing and foraging at night. Both
mornings, Sloth 3 had moved substantially in
position relative to the previous evening.

**DISCUSSION**

Across studied individuals, there was no
relationship between ambient temperature and
activity levels. Furthermore, we did not find a
significant difference in activity across individuals
in terms of cloud cover, sunlight, or general time
of day. However, since there was a significant
difference in activity between study subjects,
analyzing each individually might improve the
inferences from our results.

While we did not find a relationship between
ambient temperature and activity level in Sloth 1,
this might simply be due to the fact that we
observed almost no activity in this individual
across our two-day observation period. Sloth 1’s
lack of activity might be explained as a long
respite from its journey across multiple trees,
enshrining dozens of meters of horizontal
distance, just hours before our observation period
began. Since some species of sloth exhibit a
remarkably low foraging rate (mean food intake =
17 g/kg/day, Cliff et al. 2015), and Sloth 1 was not
observed eating any leaves after its relocation, we

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**Figure 4.** Logistical regressions of the presence of activity in individual sloths (1 = activity present, 0 = activity absent) in relation to average temperature (ºC) within observation intervals. Each dot represents the data from a 10-minute observation interval. Each graph represents the data from a single sloth. The number next to the Presence of Activity axis (1, 2, or 3) corresponds to each numbered sloth.
can assume that a lack of movement corresponded to exhaustion and lack of energy. Regardless, the results from Sloth 1 do not support that *C. hoffmanni* are facultative poikilotherms.

Although Sloth 2 also did not exhibit any change in activity tied to ambient temperature fluctuation, this lack of relationship might be further explained within the context of a different set of observations. We did not observe Sloth 2 traveling any more than a few meters each day of observation. However, since Sloth 2 appeared in locations each morning that were different from its location the previous evening, we can assume that Sloth 2 climbed, and perhaps foraged, during the night or the early hours of the morning. Furthermore, our HOBO temperature loggers revealed that temperatures decreased markedly by a few degrees Celsius each night, followed by a sharp rise in the morning (around 0600). These additional findings suggest that perhaps *C. hoffmanni* exhibit greater activity at night to warm their bodies in colder temperatures. If such is the case, then *C. hoffmanni* might behave more as homeotherms than as poikilotherms. Yet, unlike other endothermic mammals that use energy stores to generate heat internally in cold temperatures, *C. hoffmanni* might use their movement to generate body heat and keep warm at night.

Our results for Sloth 3 further support that *C. hoffmanni* behave as homeotherms in regulating their body temperature in response to ambient fluctuation. Unlike the other individuals, Sloth 3’s activity decreased with increasing temperatures. Sloth 3 did not travel a significant distance during the study period, but climbed and foraged frequently, with night movement observed directly and inferred by changes in position from evening to morning. The data from Sloth 3 seem to directly refute the hypothesis that *C. hoffmanni* might be facultative poikilotherms. Rather, the data support that *C. hoffmanni* follow a thermoregulatory scheme characteristic of other homeothermic mammals. Again, Sloth 3’s activity at night could support that nocturnal climbing and foraging might compensate for the exceptionally low food consumption rates of *C. hoffmanni* that would otherwise be necessary to maintain internal temperatures through metabolic processes.

Our results suggest that, although *C. hoffmanni* respiration and foraging patterns might be consistent with facultative poikilothermy (Costello and Rieb 2012, Kredel 1928, Britton 1938), their overall behavior is more consistent with homeothermy. Britton and Atkinson (1938) found that *C. hoffmanni* have a 1.5 °C range for body temperature. High temperatures during the day might supply enough thermal energy for *C. hoffmanni* to sleep and remain relatively inactive while retaining a consistently warm body temperature. However, since *C. hoffmanni* consume such small levels of food with such a long digestion period (6-21 days, San Diego Zoo 2009), their low energy stores alone might necessitate movement and foraging at night to maintain warmer body temperatures through kinetic activity. Further, *C. hoffmanni* are not able to shiver, so this movement might be the only way to increase body temperature when ambient temperatures decrease (San Diego Zoo 2009). We found no evidence that *C. hoffmanni* is exclusively nocturnal, for we recorded numerous instances of climbing and foraging throughout the day.

Regardless, we observed that *C. hoffmanni* moved more during the night than the day, due to the significant changes in location of numerous individuals each morning. However, this preference for night activity does not seem to be dictated by predator-prey dynamics or competition. If anything, predation risk from jaguars, other large cats, and snakes would be higher at night, and there does not appear to be a significant competitor for leaves during either time of day. Instead, *C. hoffmanni* may follow a unique scheme of homeothermy among endothermic mammals that relies on kinetic movement at night, rather than metabolizing accrued food stores, to generate heat.

Future studies could investigate additional facets of the relationship between variations in ambient temperature and *C. hoffmanni* behavior. Given our limited time and resources, we were not able to observe *C. hoffmanni* individuals overnight, when we believe they are most active. Observational data at night, when temperatures are lowest, could produce a significant logistical relationship between activity and ambient temperature across all *C. hoffmanni* individuals. Further, increasing our small sample size could enhance the inference space of our results. The population density of *C. hoffmanni* is approximately three to five individuals per hectare (La Selva Biological Station Staff, pers. comm.).
suggesting that a larger sample size would require a much larger sampling area. Tracking individuals’ location via GPS would allow measurements of travel distance by day and time of day, which could offer a better understanding of *C. hoffmanni* energy expenditure. Finally, being able to measure internal temperature of study subjects would provide significantly more information about how ambient temperature affects *C. hoffmanni* thermoregulation.

In conclusion, our observations suggest that activity patterns in *C. hoffmanni* represent behavioral homeothermy as opposed to facultative poikilothermy. While an organism may exhibit relationships between ambient temperature and respiration or metabolic rate characteristic of a poikilotherm, this does not mean that their behavior follows the same thermoregulatory trend. Thermoregulation is a fundamental facet of survival that has been under selection for millennia, and might differ in its specifics for any given species, regardless of trends within a clade. Thus, endothermic homeothermy in mammals may be expressed in different ways through various forms of energy expenditure.

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**AUTHOR CONTRIBUTIONS**

All authors contributed equally.

**LITERATURE CITED**


EFFECT OF PROTECTED AREAS ON QUEEN CONCH (LOBATUS GIGAS) POPULATION
DEMOGRAPHICS

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Abstract: Human impacts, such as overharvesting of recreationally and commercially caught species, have led to the decline of many marine species. Recent management policies that attempt to mitigate or reverse these negative effects range from broad, such as large-scale Marine Protected Areas, to specific, such as smaller-scale no-harvest zones. On Little Cayman Island, Queen Conch (Lobatus gigas) are protected by no-take zones and daily harvest limits. In 2012, Chowdhury et al. studied the effectiveness of a no-harvest “replenishment” zone in South Hole Sound, Little Cayman Island. Five years later, we have replicated their study to reassess the success of the replenishment zone. We surveyed transects in both the replenishment and harvested zones and recorded the length and thickness of each L. gigas individual, the presence or absence of a flange, and whether the animal was alive or dead (i.e. the shell was empty). We found that length and thickness of adults did not vary between zones. We also found that density and rate of harvesting were not significantly different between the replenishment and harvested zones. Finally, we found that the overall density in 2017 is one fourth the density found in 2012. This drastic drop in L. gigas population over just five years suggests that management effectiveness has decreased in success and emphasizes the need for continued monitoring and adaptation in L. gigas protection.

Key words: adaptive management strategy, Queen Conch, replenishment zone

INTRODUCTION
Coral reefs have provided ecosystem services to the people who live near them for thousands of years. Hundreds of millions of people worldwide currently rely on coral reefs and nearby marine ecosystems for food from fishing, coastal protection, and shoreline stabilization (Pendleton et al. 2016). However, because of human impact, coral reefs are in decline globally. People have adopted a variety of policies to try and mitigate or reverse human effects on reefs. Marine Protected Areas (MPAs) aim to protect whole ecosystems while more targeted approaches like no-take zones are put in place for species that are being overfished. Previous studies have shown that no-take zones can have positive effects on marine invertebrate population growth (Dumas et al. 2010). By prohibiting or limiting harvesting, these no-take “replenishment” zones can reduce overharvesting and allow for more natural population growth.

One example of a heavily harvested species is the queen conch (Lobatus gigas), which is protected in areas around Little Cayman Island. L. gigas habitat ranges from turtle grass beds to coral rubble around reefs, to deeper algal plains (Danylchuk 2003; Governali et al. 2017). Natural predators of L. gigas include turtles, nurse sharks, crabs, and lobsters, but once individuals reach a terminal length, they start increasing the thickness of their shell, reducing predation vulnerability. However, L. gigas are easy for humans to harvest at any length or shell thickness because they are slow-moving animals that live in relatively shallow water, and because they often aggregate in groups. In general, L. gigas populations across the Caribbean have experienced significant decline from human impact (Stoner 1996). Given the easy access for humans, L. gigas has been historically overharvested on Little Cayman Island. Realizing this, the Cayman Department of Environment (DoE) established a no-take zone for L. gigas in 1987 (Chowdhury et al. 2012). Current regulations set harvest limits at 10 L. gigas per boat per day or five L. gigas per person per day, whichever number is smaller (Caymanian DoE 2016).

Chowdhury et al. (2012) conducted a study to determine whether the replenishment zone in South Hole Sound, Little Cayman Island was effective in maintaining the population of L. gigas. They found that the density of L. gigas was significantly higher in the replenishment zone than in the area open to harvesting. Chowdhury et al. (2012) concluded overall that the replenishment zone was effective in protecting the L. gigas population in South Hole Sound. A common
challenge in conservation management is a lack of ongoing research and monitoring. This leads to an inability to adjust management strategies or even assess if protection is effective. Given this, we sought to replicate their study as a reassessment of the replenishment zone five years later to inform future *L. gigas* management on Little Cayman Island.

Overall, we sought to determine whether the current management strategy is having any impact on the *L. gigas* population in South Hole Sound. We began by comparing our method of distinguishing adults and juveniles to the 2012 method: we used shell thickness, whereas the 2012 study used the presence of a flange to indicate sexual maturity. If both methods are effective at identifying sexual maturity, we would expect to find no difference in the number of adults identified by shell thickness and by flange. If humans preferentially harvest large individuals to maximize the amount of meat per *L. gigas*, we would expect shell length to be longer in the replenishment zone, as the population there would not be under harvesting pressure. We would expect to find higher mortality (i.e. proportion of empty shells to live *L. gigas*) in the harvested zone, if *L. gigas* is harvested in situ and shells are dropped in the same area from which they were harvested and if *L. gigas* are harvested at a higher rate than natural mortality. We would also expect a higher proportion of empty shells to be harvested in the harvested zone. If the management strategy is succeeding, we would expect that the density of *L. gigas* in the replenishment zone would be higher than that in the harvested zone.

**METHODS**

*Data collection*

We conducted an observational field study on 19 and 20 February 2017 in South Hole Sound, Little Cayman Island. We collected data on *L. gigas* individuals and empty shells in the sand substrate between the shore and Owen Island. Half of the transects fell within the replenishment zone and half were farther west in an area open to *L. gigas* harvesting (harvested zone; Figure 1). We set up twenty 40 m by 2 m transects in both the replenishment zone and harvested zone using measuring tape and two-pound weights. The starting point of each transect was separated by about 10 meters from the adjacent transects.

![Map of sampling locations with replenishment zone shaded (Chowdhury et al. 2012).](image1)

![The shell of an adult *L. gigas* with measured dimensions and key features indicated (Collins and Harrison 2007).](image2)
Data analysis
To understand the relationship between presence or absence of flange with shell lip thickness, we compared the number of adults at each site based on flange to the number of adults at each site based on shell thickness. We then binomially labelled each individual, based on flange or shell thickness, as an adult (1) or juvenile (0) to determine the ratio (percent composition) of adults to juveniles in both the replenishment and harvested zones for further comparison.

To understand the relationship between zone type (replenishment or harvested) and the length of adults and juveniles overall and in each zone, we performed a one-tailed t-test. Then, to understand the relationship between zone and adult shell thickness, we performed a two-tailed t-test.

To calculate the mortality rate, we divided the number of dead *L. gigas* by total number of *L. gigas* found overall and in each zone. Then, to determine whether the distribution of dead *L. gigas* varied between the replenishment and harvest zones, we used a chi-square test. Within the dead *L. gigas*, we calculated the proportion of dead due to harvesting in each zone and overall. We used a chi-square test to generate a contingency table to assess the difference in proportions of *L. gigas* that died due to harvesting versus those that died naturally between zones.

To determine whether the densities of adults and juveniles in both harvested and replenished zones differed, we conducted a one-tailed t-test. Additionally, to compare our density data to data collected in 2012 using the same methods, we performed a second one-tailed t-test.

All t-tests were conducted in JMP Pro 13 and chi-square tests were conducted in Microsoft Excel.

RESULTS
Overall, we found that categorizing adults based on shell thickness produced a value of 27 adults across harvested and replenished zones, while categorizing adults based on presence of flange produced a value of 37 adults across both sites. We found no difference in the percentage of adults to juveniles (45% adults) in the replenishment zone according to thickness and flange measurements (i.e., both predictors produced the same number of adults). However, we found that the ratio of adults based on the presence of flange (59%) was much higher than the ratio of adults based on shell thickness (28%) in the harvested zone, indicating that the methods of Chowdury et al. (2012) overestimated adult abundance compared to our methods.

Shell length did not vary significantly between the replenishment zone (mean ± SE = 18.98 ± 0.66) and the harvested zone (mean ± SE = 19.71 ± 0.74) (*t* = 0.23, *P* = 0.46, df = 70). Adult shell thickness also did not vary significantly between zones (*t* = -0.65, *P* = 0.53, df = 19.10).

There was no difference in either overall mortality or harvest mortality between the replenishment and harvested zones. Of all *L. gigas* measured, 82.19% were found alive. Within the replenishment zone, 79.5% were alive and 20.5% dead. Within the harvested zone, 84.8% were alive and 15.2% were dead. There was no significant effect of zone on proportion of *L. gigas* that were dead (chi-square = 0.29, *P* = 0.59, df = 1). Of all dead *L. gigas* that were measured, 50% were harvested. In the replenished zone 57% of empty shells were harvested, while in the harvested zone 40% were harvested. There was no significant effect of zone on the proportion of dead *L. gigas* that were harvested (chi-square = 0.124, *P* = 0.72, df = 1).

There was no significant difference in density of total *L. gigas* between the harvested and replenished zones (*t* = 0.645, *P* = 0.26, df = 38). There was also no significant difference in the density of adult *L. gigas* between zones, or in the density of juvenile *L. gigas* between zones (adults: *t* = 0.15, *P* = 0.44, df = 7.25; juveniles: *t* = -0.52, *P* = 0.69, df = 17.46). Mean *L. gigas* density in 2012 (1031 individuals/hectare) was significantly higher than in this study (221.9 individuals/hectare; *t* = -5.77, *P* < 0.001, df = 58; Figure 3).

Variation in depth between transects and zones was negligible within and across our sampling areas (1 to 2 meters).
**DISCUSSION**

Overall, our results suggest that there is no difference in population between replenishment and harvested zones. There was also no difference in the adult to juvenile ratio or length and thickness between zones. The lack of difference between harvested and replenishment zones implies that management and prevention of human harvesting in the replenishment zone has not been effective in creating a different, more protective, habitat for *L. gigas*. Furthermore, there was a significant decline in density between 2012 and 2017. These results further suggest that the replenishment zone is ineffective at preserving and protecting the *L. gigas* population in South Hole Sound. While human impact is likely, observed decline in the *L. gigas* population could be due to other factors. One possibility is that changes in temperature or food availability might negatively impact recruitment, which could lead to a population decline. These potential changes could also cause *L. gigas* to move towards the more preferable coral rubble substrate (Governali et al. 2017). Either way, this reassessment of density, showing a drastic change in just five years, emphasizes the importance of collecting data to adapt and inform management decisions.

Although the proportion of empty shells that were harvested was slightly higher in the replenishment zone, this difference was not significant. One explanation for the unexpected higher proportion of harvested shells in the replenishment zone could be that shells are dropped from boats in the replenishment zone after individuals are harvested in the harvested zone. Another possibility is that harvested shells could drift, from the harvested zone into the replenishment zone, however, the current in South Hole Sound usually runs from the replenishment zone to the harvested zone. One island resident who lives near the replenishment zone has observed boats from Cayman Brac poaching *L. gigas* from the replenishment zone (Joy Mulholland pers. comm.). If this is the case, it could explain the drastic population decline from 2012.

The discrepancy in methods for identifying adults between 2012 and this study could have influenced our comparison of results, but this discrepancy seems inconsequential in the data we compared. More individuals were considered adults using flange as an indicator of sexual maturity than using shell thickness. However, this difference in measurement does not impact results.

![Figure 3. Density of *L. gigas* in the entire sampling area (both replenishment zone and harvested zone) in 2012 and 2017.](image)
of overall density. Additionally, it does not impact
the comparison of the proportion of adults and
juveniles between zones. If researchers in 2012
had used thickness as an indication of sexual
maturity, they would have found even less of a
difference between the proportion of adults and
juveniles, a result closer to that which was found
in our study. Age distribution does not seem to be
determined by harvesting pressure as there was no
difference in shell thickness between the
replenishment and harvested zones.

When compared to the results of the 2012
study, our findings emphasize the importance of
continued data collection to adapt management
strategies. If the DoE knows that this
replenishment zone has become less successful
over the last five years, it might be able to
implement new conservation strategies. Increasing
the size of the replenishment zone could better
buffer the *L. gigas* populations from harvested
zones. Additionally, enforcing protections to
reduce poaching in the replenishment zone could
better protect the *L. gigas* population. If these
changes in protection are not supported by the
community nor enforced by the DoE, however, the
population will continue to decline.

Ultimately, to adapt to increasing human impacts
on these harvested species, management decisions
need to be informed by continued monitoring of
the ecosystem.

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REEF CORAL-ATIONS: SPECIES INTERACTIONS AFFECTING CORAL COVER ON LITTLE CAYMAN

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Abstract: Coral reefs are one of the most diverse ecosystems on earth. Reefs provide direct economic and ecosystem services to over half a billion people. Previous research has shown that degraded coral reefs often undergo phase shifts in which they become macroalgae-dominated. Factors like predation and herbivory can be determinants of these phase shifts by influencing the interactions between coral and algae. Evidence exists for correlations between herbivory by algivorous fish and increased coral growth rates. Predation by piscivores may reduce the density of algivores, thereby increasing the competitive advantage of macroalgae over coral. We examined these potential controls on macroalgal and coral cover for two reefs on Little Cayman Island: an unprotected reef at Thornton’s flats and the Marine Protected Area (MPA) behind Central Caribbean Marine Institute (CCMI). We estimated percent macroalgal and coral cover using photo analysis of transects from each site. Fish density and diversity surveys were also conducted at each reef. We found no significant correlation between percent algal cover and percent coral cover. Additionally, there was not a significant correlation between piscivore density and algivore density. However, there was a significant negative correlation between percent algal cover and the density of algivorous fish. The MPA had a significantly higher density and diversity of fish. Our results provide evidence suggesting that herbivory by algivorous fish is the most likely mechanism controlling algal cover. If MPAs in general maintain higher algivore densities, as in our study system, then herbivory may be greater and algal cover reduced in these areas.

Key words: macroalgae, herbivory, marine protected parks

INTRODUCTION

Coral reefs are some of the most biologically diverse ecosystems on earth and provide direct economic and ecosystem services to over half a billion people (Sheppard 2014). Reef systems are structured by species interactions between trophic levels, from primary producers such as macroalgae to top piscivorous predators (Brooker et al. 2016). A variety of anthropogenic factors including climate change, fishing pressure, and nutrient enrichment contribute to coral reef decline worldwide. Previous research has shown that degraded reefs undergo phase shifts from coral-dominated to macroalgae-dominated systems (Hughes 1994). The most immediate impact of these phase shifts is a loss of fish biodiversity and changes in community structure (Munday et al. 2008). On a global scale, anthropogenic climate change is driving reef decline through increases in temperature and ocean acidification (Hoegh-Guldberg et al. 2007), which result in phase shifts to macroalgae-dominated systems. On a smaller scale, fishing activity at different trophic levels has been shown to impact levels of predation and herbivory within the ecosystem (Mumby et al. 2006), which could have cascading effects on lower trophic levels. Management policies that limit human activities in an attempt to address these impacts have been shown to increase the fish biomass and decrease macroalgal biomass within MPAs (Mumby et al. 2006).

Species interactions in coral reefs can affect coral survival. Macroalgae can directly negatively impact corals through interspecific competition in the absence of herbivory (Rasher and Hay 2010). Thus, in reefs with low densities of algivorous fish, macroalgal cover can be high relative to coral cover. Higher abundance of herbivorous fish has been shown to result in greater coral growth in certain seascapes (Brandl et al. 2014), perhaps because herbivorous fish provide an effective control on macroalgal growth (Burkepile and Hay 2006). Predation by large piscivores on herbivores can lead to reductions in grazing (Mumby et al. 2006).

We investigated the relationships between substrate composition (coral and macroalgal cover) and algivorous and piscivorous fish densities to test whether competition, herbivory, and predation might mediate the relationship between coral and macroalgae. If interspecific competition controlled the relative coral and
macroalgae cover on the reef, we would observe a negative correlation between the percent cover of the two substrates. If herbivory, in addition to competition, mediated coral-algal interactions, we would observe a negative correlation between macroalgal cover and algivore density and a positive correlation between coral cover and algivore density. Alternatively, if herbivory controlled macroalgal growth rather than competition, we would observe no correlation between coral cover and algivore density and a negative correlation between macroalgal cover and algivore density. If predation mediates coral-algal interactions in combination with herbivory and competition, we would observe negative correlations between piscivore density and both algivore density and coral cover, and a positive correlation between piscivore density and macroalgal cover.

We investigated the effects of human management on reefs by comparing total fish density, algivorous and piscivorous fish density, number of fish species, and macroalgal and coral cover at two sites with different levels of human activity: a marine protected area (MPA) and an unprotected area. If there was fishing pressure on piscivorous fish outside of the MPA, we would observe a higher density of fish overall within the MPA. We might also observe a greater number of fish species within the MPA. Finally, we might observe higher coral cover and lower macroalgal cover within the MPA, especially if herbivory impacts coral-algal interactions. Our study examines the effects of coral-algal competition, fish biomass and diversity, and the effects of human management on coral and algal cover on the reef.

METHODS
We compared percent of coral and macroalgal cover as well as algivorous and piscivorous fish densities between Thornton's Flats and the MPA behind the Central Caribbean Marine Institute (CCMI) on Little Cayman Island. Thornton’s Flats is located on the south side of the island and fishing is allowed, while the MPA is protected from fishing and located on the north side of the island. On February 23rd and 24th, we ran 30 x 2 m transects along each reef (n=7 for Thornton’s Flats; n=4 for the marine park). Two snorkelers swam each transect at a consistent pace and recorded the number and taxonomic group of fish present within the transect. The identified fish were sorted into algivorous and piscivorous groups. Total fish, including those that were neither algivores nor piscivores, were counted to determine fish densities.

We then photographed five 0.25m² quadrats along each transect. The distance of each quadrant along the transect was determined using a random number generator. The percent of each photographed quadrant covered by coral and algae was analyzed using image-editing software (ImageJ). Each image had an approximate resolution of 2500 by 2500 pixels. Using the freehand tracing tool, algae and coral present in the image was manually traced and analyzed for a measure of area.

We calculated total fish density for each transect by dividing the number of fish counted by the area of each transect (60 m²). Similarly, we calculated piscivore and algivore density by dividing the number of each feeding guild by the transect area. We counted the number of different fish within each transect (to the level of the genus), and used this number as a proxy for fish diversity.

In JMP Pro v.13, we used a pairwise correlation matrix (Pearson product-moment correlation) to assess correlations between fish density, fish diversity, algivore density, piscivore density, the percentage of algal cover, and the percentage of coral cover across both sites. For these analyses, we excluded a single outlier from the density of piscivores and algivore data. Additionally, we used a t-tests to test for the effect of site on fish density, fish species diversity, algivore density, piscivore density, the percentage of algal cover, and the percentage of coral cover. All percentage data were arcsine transformed for this analysis.

RESULTS
There were significant correlations between fish densities and algal cover, but not coral cover. The percentages of algal and coral cover were not significantly correlated across sites ($r = -0.45$, $P = 0.17$, df = 10; Table 1). Algivore density was significantly negatively correlated with percentage of algal cover ($r = -0.69$, $P = 0.02$, df = 10; Fig. 1), but not significantly correlated with percentage of coral cover ($r = 0.20$, $P = 0.55$, df = 10).
Piscivore density was not significantly correlated with algivore density \( (r = 0.065, P = 0.48, \text{df} = 19) \). Piscivore density was also not significantly correlated with percentage of algal \( (r = -0.51, P = 0.11, \text{df} = 10) \) or coral cover \( (r = 0.14, P = 0.68, \text{df} = 10) \).

The algivorous fish observed included damselfish, gobies, tangs, sergeant majors, and parrotfish. The piscivorous fish observed included basslets, snappers, and bar jacks. Gobies and sergeant majors were included in both categories because the fish species included in those groups are omnivorous, eating both algae and other fish. We also observed many fish that were not grouped in either category but were included in measurements of diversity and total density, including grunts, squirrelfish, porcupine fish, goatfish, wrasse, and pigfish.

Mean fish diversity was significantly higher at CCMI \( (7.00 \text{ taxa/m}^2) \) than at Thorton’s Flats \( (3.86 \text{ taxa/m}^2) \) \( (t = -2.21, P = 0.043, \text{df} = 4.31; \text{Table 2}; \text{Fig. 2}) \). Mean fish density was also significantly higher at CCMI \( (1.72 \text{ fish/m}^2) \) than at Thorton’s Flats \( (0.57 \text{ fish/m}^2) \) \( (t = 5.60, P = 0.002, \text{df} = 4.69) \). Algivore density was significantly higher at CCMI \( (24.53 \text{ algivores/m}^2) \) than at Thorton’s Flats \( (10.86 \text{ algivores/m}^2) \) \( (t = -3.42, P = 0.014, \text{df} = 3.99) \). Piscivore density was not significantly different between sites, but there was a trend toward higher piscivore density at CCMI \( (3.42 \text{ piscivores/m}^2) \) than at Thorton’s Flats \( (0.71 \text{ piscivores/m}^2) \) \( (t = -2.15, P = 0.055, \text{df} = 3.41) \).

The percentage of algal cover was significantly higher at TF \( (31.22\%) \) than at CCMI \( (10.32\%) \) \( (t = 5.24, P < 0.001, \text{df} = 7.89) \), but the percentage of coral cover was not significantly different between sites \( (t = -1.35, P = 0.13, \text{df} = 3.1) \).

**Table 1.** Correlations between substrate and fish density

<table>
<thead>
<tr>
<th>Variable</th>
<th>By Variable</th>
<th>Correlation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Coral</td>
<td>% Algae</td>
<td>Insignificant</td>
<td>0.17</td>
</tr>
<tr>
<td>Algivore density</td>
<td>% Coral</td>
<td>Insignificant</td>
<td>0.55</td>
</tr>
<tr>
<td>Algivore density</td>
<td>% Algae</td>
<td>-0.69</td>
<td>0.02</td>
</tr>
<tr>
<td>Piscivore density</td>
<td>Algivore density</td>
<td>Insignificant</td>
<td>0.38</td>
</tr>
</tbody>
</table>

**Table 2.** Outputs of t-tests investigating the effect of site on fish and substrate measures

<table>
<thead>
<tr>
<th></th>
<th>CCMI</th>
<th>TF</th>
<th>t value</th>
<th>df</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish diversity</td>
<td>7</td>
<td>3.42</td>
<td>-2.21</td>
<td>4.31</td>
<td>0.043</td>
</tr>
<tr>
<td>Log fish density</td>
<td>1.72</td>
<td>0.5738</td>
<td>5.6</td>
<td>4.69</td>
<td>0.002</td>
</tr>
<tr>
<td>% Algae</td>
<td>10.32</td>
<td>31.22%</td>
<td>5.24</td>
<td>7.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>% Coral</td>
<td>16.18</td>
<td>32.30%</td>
<td>-1.35</td>
<td>3.14</td>
<td>0.13</td>
</tr>
<tr>
<td>Algivore density</td>
<td>24.53</td>
<td>10.86</td>
<td>-3.32</td>
<td>3.99</td>
<td>0.014</td>
</tr>
<tr>
<td>Piscivore density</td>
<td>3.41</td>
<td>0.71</td>
<td>-2.15</td>
<td>3.41</td>
<td>0.055</td>
</tr>
</tbody>
</table>

![Graph](image.png)
Figure 2. (a) The mean number of observed fish taxa was significantly higher at CCMI than at Thornton's Flats (TF). (b) Fish density was significantly higher at CCMI than at TF. (c) Algivore density was significantly higher at CCMI than at TF. (d) Piscivore density was not significantly different between sites. (e) Percent algal cover was significantly higher at TF than at CCMI. (f) Percent coral cover was not significantly different between sites.
DISCUSSION

There was no significant correlation between percent coral cover and percent algal cover. These results contradict previous literature, which suggests that there is interspecific competition for resources between algae and coral in marine ecosystems because of light availability, and substrate area (McCook et al 2001). There have also been documented allelopathic interactions between coral and algae, in which algal toxins prevent the growth of coral in proximity (Rasher and Hay 2010). However, in our study sites we observed very low coral cover overall, and many of the areas surveyed showed a large amount of uncolonized dead coral. This suggests that the reason we observed no correlation between the percent coral cover and algal cover is because they have not reached high enough densities for competition, and the percent cover has more to do with recruitment than competition.

We found that algal cover was negatively correlated with the density of algivorous fish. This suggests that herbivory by algivorous fish may act as a top-down control on algal growth. On the other hand, the negative interaction between algivore density and algal cover could be explained by reef fish behavior. Dixson et al. (2014) showed that fish larvae across species were attracted to chemical cues in water from reefs in MPAs when compared to water from non-MPA reefs. Fish larvae might be more attracted to less degraded reefs, and therefore reefs with less algae, while areas with more algae would attract fewer algivores.

Although one would expect higher coral cover as algivore density increased if there was interspecific competition between coral and algae, there was no observed correlation between coral cover and algivore density. This might be due to the lack of interspecific competition observed in the study system as explained above.

We found that piscivore density is not significantly correlated with algivore density across transects. This contrasts with the prediction under a trophic cascade model, in which piscivore density would be negatively correlated with algivore density. An increase in the piscivore population would presumably lead to a decrease in algivore population due to predation, which, in turn would lead to an increase in algal cover due to herbivory release. However, this is not the correlation that we observed. Predation therefore does not seem to be structuring the community, especially the composition of macroalgal and coral cover. This follows our finding that there was no significant correlation between piscivore density and algal cover or coral cover. This suggests that top-down effects from the piscivore trophic level do not control the system and differences in algal cover are not a result of predation.

We observed stark differences in the fish communities between sites: CCMI had higher fish density and diversity. Since the herbivory mechanism is the one most supported by our results, it could be that the fishing regulations in this MPA are maintaining a higher algivore density, thereby reducing algal cover. Conservation efforts, even in an area of low fishing pressure, as in Little Cayman, are likely quite important for maintaining the integrity of coral reefs.

According to our study, herbivory seems to control macroalgal densities. Though we did not test for causation, the strong negative correlation between algivore density and percent algal cover suggests that the algivorous fish are grazing on the algae, resulting in lower algal cover. The comparison of the MPA to the unprotected site further supports this, because algal cover was significantly lower in the MPA, while algivore density was significantly higher. As the main regulation in the MPA on Little Cayman is no-take of any marine life, this difference between sites suggests that the increased algivore density and resulting herbivory is driving the lower algal cover. This study therefore demonstrates the importance of herbivory in maintaining low macroalgal growth. Even in this system, we found the MPA to have a higher diversity and fish density, suggesting a more vibrant marine reef community that could potentially provide ecosystem services such as decreasing algal growth. Our study therefore demonstrates the importance of conserving algivorous fishes in the reef system in Little Cayman, and potentially elsewhere where herbivory is controlling macroalgal growth.

Additionally, we found that species interactions at three trophic levels (competition, herbivory, and predation) were not structuring coral cover in this system, as there were no correlations between coral cover and any of the
variables we tested. Further investigation is required to determine what mechanism dictates coral cover, such as larval recruitment or nutrient enrichment. Other future studies might also investigate coral and algal diversity and its relationship with the community structure of these systems, especially when comparing MPA’s with unprotected areas.

Herbivory and algae seem to be closely related, which is important information for future management of coral reefs and other marine environments in which increasing macroalgae as a result of human intervention can be detrimental. To preserve the ecosystem services provided by these areas, managers should focus on conserving algivorous fish to keep macroalgae populations controlled.

LITERATURE CITED


EFFECT OF FISH TRAFFIC ON TERRITORIAL BEHAVIOR IN COCOA DAMSELFISH
(STEGASTES VARIABILIS)

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Abstract: Animal behavior is limited by tradeoffs between resource acquisition and energy expenditure. These tradeoffs vary with context, so it is advantageous for organisms to be flexible in their behaviors. Optimal decision theory asserts that decision-making is influenced by environmental conditions, such as competition for food resources. Cocoa damselfish (Stegastes variabilis) might be impacted by such pressures to maximize energetic efficiency, as the fish maintain and defend algal gardens that serve as a food source. This tradeoff in energy expenditure might be affected by the presence of other fish. For example, with higher fish traffic, S. variabilis might defend more and feed less. To investigate this tradeoff, we measured traffic and frequency of attacks (defense against surrounding fish) and pecks in 45 juvenile S. variabilis individuals across two reefs off of Little Cayman Island. Fish traffic had a significantly negative effect on the frequency of pecks and a significantly positive effect on frequency of attacks. Across S. variabilis individuals, when controlling for traffic level, the frequency of attacks increased with the frequency of pecks, suggesting that some individuals were more active than others. Traffic of both conspecifics and heterospecifics produced similarly significantly positive effects on the frequency of attacks on those two categories of intruders. Our study contributes to an understanding of how energetic tradeoffs influence territorial behavior.

Key words: algal turf, Caribbean, competition, coral reef

INTRODUCTION
Organisms are limited in their behavior by their ability to acquire energy (Sinervo 1997). Individuals cannot expend more energy than their resources permit, and thus energetic tradeoffs often affect that individual’s expressed behavior (Agrawal et al. 2010). It is advantageous for an organism to behave in a way that minimizes the costs of these tradeoffs and maximizes energetic efficiency. However, the tradeoffs faced by a species are often not constant, but instead might be context-dependent. Optimal decision theory postulates that an individual will make decisions based on thresholds in environmental conditions such as food resources and proximity of intruders (Lepora 2016, Parker and Smith 1990).

Cocoa damselfish (Stegastes variabilis) maintain territories containing gardens of algae that they aggressively defend from intruders to reduce grazing pressure (Deloach & Humann 2003, Hata and Kato 2002). Different damselfish species display different strategies for the maintenance of their algal gardens, which are cultivated for a stable supply of energy. Some damselfish species, such as S. nigricans, pick out and remove indigestible algae in order to maintain relatively small algal gardens that are more easily defended against other herbivorous fishes (Hata and Kato 2004). Conversely, other damselfish species, such as S. obreptus, maintain their algal gardens less diligently and instead rely more heavily on defending their territories from intruders (Hata and Kato 2004).

There is also evidence that damselfish display different defensive strategies depending on the species of the invader. It seems that while S. variabilis completely exclude conspecifics from all three main areas of their territories (core areas, peripheral areas, and paths), they do not completely exclude heterospecifics (Gronell 1980). Higher rates of aggression towards conspecifics have similarly been observed in other damselfish species, including the Brazilian damselfish (S. fuscus) (Osorio et al. 2006). It is plausible that S. variabilis experience tradeoffs associated with their ability to devote energy toward algal maintenance versus attacks (defense) against surrounding fish, and this tradeoff might be affected by the presence of other fish.

In this study, we explore how the traffic of fish near a juvenile S. variabilis affects its pecking and attack behaviors. We hypothesize that
increased presence of other fish will warrant more attacks, and that a tradeoff between algal maintenance and defense will therefore necessitate lower algal maintenance. Thus, we predict that the frequency of _S. variabilis_ attacks will increase and _S. variabilis_ algal maintenance will decrease with the number of encounters it has with other fish. We predict this trend to be most pronounced among encounters with conspecifics. While the frequencies of traffic, defensive behavior, and pecking behavior may vary between study sites, we would expect to see the predicted trends in behavior proportional to traffic across sites.

METHODS

Field methods

We observed the effect of fish traffic on the rates of pecking and attacking for _S. variabilis_ in the sandy benthos near patch reefs. We identified 45 juvenile _S. variabilis_ territories on Little Cayman near patch reefs in Thornton’s Flats and in the back reef of the Central Caribbean Marine Institute (CCMI). We observed each focal fish’s pecks and attacks for five minutes. We also recorded the number of times a fish (conspecific or heterospecific) came within 15 cm of the focal fish, the number of times the focal fish pecked at algae or algal epiphytes, and the number of times the focal fish attacked other fish (conspecific or heterospecific). We defined an attack as a rapid movement toward another fish and subsequent fleeing of the other fish.

While pecks and attacks were not the only two activities observed, they were the only behaviors that we measured. _S. variabilis_ also spent a significant amount of time patrolling their territory and hiding in or under rocks. We occasionally observed _S. variabilis_ chasing intruders out toward the periphery of their territories showing increased aggression toward conspecifics. Some chases led to long interactions, and each strike was considered a distinct attack.

Statistical methods

We performed linear regressions to determine the effects of total fish traffic, behavior type (peck or attack), and their interaction on behavior frequency. To determine the relationship between the behaviors, we performed a linear regression between pecks per passing fish and attacks per passing fish. We performed a linear regression to determine the effect of the proportional abundance of _S. variabilis_ traffic (number of proximal _S. variabilis_ as a proportion of total traffic; logit-transformed) on the frequency of pecks. We performed a generalized linear model with a Poisson error distribution and an identity link function to determine the effect of conspecific or heterospecific traffic on attack rate on other conspecifics or heterospecifics, respectively. To see if the location of data collection was important, we performed an ANOVA to determine the effect of location of data collection on pecks and attacks relative to traffic.

RESULTS

Pecking and Defensive Behavior

Across trials, the number of pecks decreased significantly with increasing traffic (slope = -0.549 ± 0.17, _P_ = 0.003, _r^2_ = 0.19; Fig. 1). Conversely, the total number of attacks towards other fish significantly increased with an increase in traffic (slope = 0.146 ± 0.03, _P_ < 0.001, _r^2_ = 0.30). The interaction between frequency of pecking or attacks and traffic was significant (F<sub>3,86</sub> = 15.5, _P_ < 0.001).

![Figure 1. Effect of traffic intensity on the frequency of pecks and attacks.](image-url)

Across individual fish, when the frequencies of both pecks and attacks were adjusted for traffic, an increase in pecks corresponded to a significant increase in attacks (slope = 0.031 ± 0.01, _P_ = 0.015, _r^2_ = 0.13; Fig. 2).
Although all observed *S. variabilis* were relatively the same size and were found in similar reef environments, we found a significant difference in the number of pecks and attacks, adjusted for traffic, between the two sites ($F_{1,43} = 9.46, P = 0.004; F_{1,42} = 27.1, P < 0.001$ respectively). The *S. variabilis* within the back reef site had higher mean frequencies of pecks and attacks ($4.32 \pm 0.49, 0.398 \pm 0.05$ respectively) than those of the Thornton’s Flats site ($0.627 \pm 0.51, 0.184 \pm 0.05$ respectively).

**Traffic Composition**

During our study, we noted that the majority of heterospecific traffic comprised of slippery dicks (*Halichoeres bivittatus*), with various surgeonfish (family Acanthuridae) juveniles comprising the second largest group. Other species of larger fish, as well as blenny (suborder Blennioidei) species and even mantis shrimp (order Stomatopoda), were seldom observed in close proximity to *S. variabilis*.

**DISCUSSION**

Our results indicate that *S. variabilis* displays behavioral plasticity, changing its pecking and defensive behaviors based on territorial context. The frequency of pecks decreased and the frequency of attacks increased, with increasing traffic. These results could be explained by optimal decision theory; more fish within a given territory might result in greater competition for resources, necessitating more frequent defense. The observed increase in attacks with increased traffic might improve the ability of *S. variabilis* to retain a healthy standing stock of algae. Similarly, increasing pecking behavior with decreased traffic might improve *S. variabilis’* ability to maintain their algal patches or acquire energy when their territory is not at risk.

Since *S. variabilis* did not divide their time solely between pecking and attacking, these behaviors were treated independently of one another in the previous analysis. When the relationship between pecks and attacks relative to traffic was analyzed, we found that *S. variabilis* that fed more also defended against intruders more frequently. It is possible that this positive correlation between pecks and attacks is due to differences in baseline activity levels between fish. However, this result could be explained from an
energetic perspective: the more a fish defends its territory, the more energy would be expended and the more it would need to feed or maintain its resources to recuperate. Fish that feed more in their territories might also have higher quality food resources that attract competing herbivores, requiring increased territorial defense. However, our study did not take into account the quality of each territory or any other factors that might affect activity levels, such as body size. Further examination of the relationship between S. variabilis peck rate and algal quality and quantity in a given territory would better contextualize this observation.

Our data support the hypothesis that S. variabilis change their behavior based on the number of fish present in their territory. However, we found that increases in both conspecific and heterospecific traffic through a territory produced similar increases in attack rate. This is inconsistent with research reporting that damselfish more rigorously defend their territories against conspecifics than against heterospecifics (Gronell 1980). This might be because we only studied juvenile S. variabilis. In adults, territories become more important for mating because the presence of conspecifics presents a threat not only to food resources, but also to potential mates. Both conspecifics and heterospecifics might pose a similar threat to food resources for juvenile S. variabilis. Because our subjects could not yet compete for mates, there might have been no need for additional aggression towards conspecifics.

Although we found a significant difference in relative pecks and attacks between sites, these results are not necessarily indicative of a variation in fish behavior due to the site. For example, since our data were collected at different locations during the morning (Thornton’s Flats) and afternoon (CCMI back reef), variation in the data could be explained by time of day. Further, while initially testing our methods, we noticed a high frequency of pecks in the afternoon at Thornton’s Flats, so anecdotal evidence might suggest that these fish are more active feeders later in the day. Alternately, it is possible that differences in site, such as differences in algal abundance or fish density, might have contributed to the behavioral differences observed between sites. A concurrent study found lower algal densities, but higher algivorous fish densities, in the CCMI back reef compared to Thornton’s Flats (Ahronian et al. 2017). A higher fish density in the back reef could produce a higher pressure for territorial defense and subsequent energy acquisition in S. variabilis, explaining the higher frequencies of pecks and attacks found at this site. Further study is required to determine whether time of day or site affects pecking and attack rates.

Territorial S. variabilis juveniles appear to balance territory defense and algal maintenance depending on the surrounding fish. These two behavioral metrics play vital roles in S. variabilis energy acquisition, and balancing the energetic tradeoffs associated with each is therefore requisite for survival. Our study offers further understanding of how energetic tradeoffs influence the context-dependency of territorial behavior in marine fish and might provide additional context for future studies of territoriality in other ecosystems.

ACKNOWLEDGEMENTS
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LITERATURE CITED


SUBSTRATE PREFERENCE IN QUEEN CONCH (LOBATUS GIGAS)

FRANCESCA C. GOVERNALI, GABRIELA J. LOWRY, LEIGH M. MOFFETT, AND EMILY B. OKUN

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Abstract: On small islands such as Little Cayman Island, protecting marine resources is integral to both the islands’ inhabitants and the ecosystems that surround it. Marine Protected Areas (MPAs) are designated zones where human activity is restricted for the preservation of natural resources. However, protecting certain species where individuals change habitat preference at different stages in their life is challenging. Queen conch (Lobatus gigas), a common gastropod found throughout the Caribbean, is a widely-harvested species throughout its range. Due to some overharvesting, restrictions and MPAs have been put in place to protect L. gigas. These gastropods have been shown to change substrate preference over its life. We studied a population of L. gigas in the marine replenishment zone of South Hole on Little Cayman Island with an observational field study to determine if they exhibit substrate preference by recording the abundance and age of individuals across four substrate types: turtle grass, sand, sandy coral rubble, and vegetated coral rubble. The highest abundance overall, and highest abundance of adults, of L. gigas was found in the vegetated rubble, suggesting that L. gigas prefers this substrate. This clear preference provides further guidance for conservation efforts and could lead to more successful protection of L. gigas on Little Cayman Island.

Key words: Lobatus gigas, Marine Protected Areas, substrate preference

INTRODUCTION

Marine Protected Areas (MPAs) are designated zones of aquatic habitat where human activity is restricted or banned for conservation purposes. These areas can safeguard populations by preventing over-harvesting and serving as reproductive reserves that can help repopulate harvested areas. However, in order to be effective in aiding conservation, MPAs need to be designed with the unique ecology and life history of the protected species in mind. Organisms often utilize a variety of habitats within their ranges during different phenological and ontological stages. Their habitat preference often depends on their specific resource needs at that stage in life. For example, entire local populations of Nassau Grouper (Epinephelus striatus) come together once a year for group spawning events. It is more effective for the long-term sustainability of the fishery to ban harvesting from the area where this vulnerable yet essential reproductive event takes place than to attempt to protect solitary fish throughout their range during other times of year (Semmens pers. comm.). Varying habitat preference presents an additional challenge to management because it tends to cause aggregations. Uneven distribution of individuals increases the difficulty of collecting accurate information on species density and abundance by introducing sampling error to commonly-used survey techniques. This further compounds conservation problems because decisions about sustainable management are fundamentally based on such population estimates, and because aggregated groups are more susceptible to harvest exploitation. For example, the Caribbean spiny lobster (Panulirus argus) – one of the most important Caribbean fisheries – expresses a strong preference towards aggregation in its juvenile stage. This tendency to gather in the same area has major conservation implications because it focusses harvest pressure on social juveniles, and has already had measurable impacts on P. argus populations (Childress et al. 2015). Thus, to sustainably manage a fishery, it is essential to understand a species’ habitat preference and how it might cause shifting aggregations across seasonal and ontological timescales.

The tropical marine gastropod Lobatus gigas (Queen conch) is an important fishery in the Caribbean, yet their populations are declining due to overharvesting. The Little Cayman Department of the Environment has designated certain areas around the island as “replenishment zones” where harvest of L. gigas is not allowed. However, they were placed without information on potential microhabitat preferences or aggregation dynamics. Strategically placing replenishment zones in areas...
with substrates that are most used by, and beneficial to, *L. gigas* could better safeguard their populations.

Nuances of their ecology and life history suggest that *L. gigas* might undergo ontological shifts in substrate usage that would affect their distribution and aggregation. A wide variety of species, including turtles, nurse sharks, crabs and lobsters, predate these algal herbivores in their juvenile stage while their shell is still growing longer. Once they reach their terminal length they stop growing in length and start thickening their shell, decreasing their vulnerability to predation. *L. gigas* also undergo ontogenetic shifts in habitat use as they age. Juveniles occupy a variety of benthic substrates but have demonstrated a preference for turtle-grass beds (Danylchuk et al. 2003). Adults require sandy substrates for reproduction because females attach their egg sacs to the sand (Aranda et al. 2014). Overall, it is unclear how these apparent preferences at varied life stages shape their typical distribution.

Based on past observations, we hypothesize that *L. gigas* abundance will differ across benthic substrates. Additionally, both adult terminal shell length and age class structure will differ across substrates. If the need for protection from predators shapes juvenile *L. gigas* substrate use, then we expect that more *L. gigas* juveniles will be found in turtle-grass, which could offer visual cover. If the need to be well located for reproduction drives adult substrate use, then the highest proportion of reproductive adults will be on sand (Aranda et al. 2014). On the other hand, if substrate use is primarily driven by foraging needs then the highest density of *L. gigas* of both age classes will occur in vegetated coral rubble, which may provide the ampest food sources. Following from this, since vegetated rubble substrate has the most macroalgae, then we would expect the mean shell length of adult conch to be longest in this substrate because the abundance of food resources would allow them to grow longer in their juvenile stage. Given that we will sample in a replenishment zone, we do not expect to find any harvested conch shells among dead individuals.

**METHODS**

We conducted an observational field study on 19 and 23 - 24 February 2017 in South Hole and near Owen Island on Little Cayman Island. We collected data on *L. gigas* individuals and empty shells in four substrate types: turtle grass, sand, sandy rubble, and vegetated rubble. The turtle grass substrate consisted of mostly turtle grass (*Thalassia testudinum*) on top of a layer of sand. The sand substrate was fine sand with occasional vegetation growth. Both the sandy rubble and...
Table 1. Variables measured for each substrate type.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Abundance</th>
<th>Mean Adult Length (SE)</th>
<th>Proportion Adult</th>
<th>% Harvested (Total)</th>
<th>% Harvested (Dead)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veg. Rubble</td>
<td>151</td>
<td>20.6 (± 0.24)</td>
<td>0.62</td>
<td>23</td>
<td>68</td>
</tr>
<tr>
<td>Sandy Rubble</td>
<td>85</td>
<td>21.6 (± 0.26)</td>
<td>0.28</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>Sand</td>
<td>27</td>
<td>18.8 (± 0.39)</td>
<td>0.11</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Turtle Grass</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>4</td>
</tr>
</tbody>
</table>

Total: 267

Note: Mean adult length variable excludes empty/dead shells.

vegetated rubble substrates were part of an area with coral rubble. Sandy rubble contained mostly coral rubble with a significant amount of sediment and less than 10% vegetation growth. The vegetated rubble substrate consisted of coral rubble with a around 60% growth of macroalgae. Variation in depth (1 to 2 meters) was negligible within and across our selected areas of substrate. Each substrate area fell within the replenishment zone in South Hole and around Owen Island (Figure 1). We set up twelve 20 meter by 2 meter transects in each of the four different substrate types using measuring tape and two-pound weights. The starting point of each transect was 10 meters east from the previous transect. Two observers swam the length of each transect looking for L. gigas individuals. When a shell was found within one meter of the transect line on either side we measured shell length, shell lip thickness, and whether the individual was dead or alive (Figure 2). If the individual was dead, we recorded the presence of a harvest hole to determine if the conch was harvested by humans or died of natural causes. Shell lip thickness was used as a proxy for individual age. Individuals with shell lip thickness greater than 9mm were considered adults for data analysis purposes (Mueller 2013). The sand substrate transects were completed on 19 February 2017 as part of data collection for a broader project.

We tested the relationship between substrate type and L. gigas adult versus juvenile abundance with a chi-square test. Additionally, we conducted a one-way ANOVA to understand the relationship between substrate type and mean adult length. The ANOVA analysis was conducted in JMP Pro 13 and the chi-square test was conducted in Microsoft Excel.

RESULTS

We collected data on a total of 261 L. gigas shells. The highest number of L. gigas (including deceased individuals) was found in the vegetated rubble substrate, followed by sandy rubble, then sand, and finally, turtle grass (Table 1, Figure 3). Density of individuals by substrate was 3146 ind./ha in vegetated rubble, 1708 ind./ha in sandy rubble, 500 ind./ha in sand, and 83 ind./ha in turtle grass. There was a significant difference between adult length across substrate types with the longest individuals found in sandy rubble ($t_{2,57} = 4.47, P = 0.02$; Table 1).

There was a significant effect of substrate on the distribution and abundance of individuals by age. The vegetated rubble had 52 more adults than juveniles while the number of juveniles exceeded the number of adults in all other substrates (Table
Furthermore, adults and juveniles were not randomly distributed across substrate types (chi-square = 2.46, $P < 0.001$, df = 3).

Forty-nine percent of all shells measured did not contain a live animal. Nineteen percent of all shells (including empty shells) had been harvested. Of all empty shells, 40% were harvested while the remaining 60% of shells were dead but not harvested.

Thirty percent of all shells appeared to have died from natural causes. Adults comprised 74% of harvested shells across all substrates. Twenty-three percent of shells found in vegetated rubble and 16% of shells found in sandy rubble had been harvested (Table 1).

**DISCUSSION**

Overall, there was a significant difference in both *L. gigas* total abundance and age distribution between substrates. Fifty-five percent of total *L. gigas* occurred in the vegetated rubble substrate suggesting that there is indeed preference for one substrate over others. Sandy rubble had the second highest abundance of individuals, with 29% of total *L. gigas* measured. The coral rubble areas may provide a combination of predator protection and resources that allow *L. gigas* to be successful. We observed that *L. gigas* shells in these substrates, especially vegetated rubble, were similar in size and shape to rubble and were covered in algal epiphytes that made them difficult to distinguish from their surroundings. As herbivores, *L. gigas* may be more attracted to the algae on coral rubble than the lesser available food resources in the turtle grass and sand substrates. As described by Aranda et al. (2014), the sand substrate is most preferred by *L. gigas* for reproduction. The lower abundance of individuals in the sand substrate during our study might be because we did not collect data during the reproductive season (March-November; DCNA 2014). Our study indicates that the need to be well located for reproduction does not determine adult *L. gigas* substrate preference during other times of year. Juveniles may move from the turtle grass to the sand as they grow larger. However, given the proximity of rubble substrates in this system, it seems individuals would not stay in the sand substrate after reproduction given the benefits of rubble substrates.

Adult length varied between substrate types, with the longest individuals found in the sandy rubble substrate. Even though thickness is used to determine age of *L. gigas*, this difference in length may show a trade-off for individuals between the two types of coral rubble substrates. While the vegetated rubble provides more food and predator protection because of greater algal abundance and camouflage, the sandy rubble substrate might provide a different benefit that allows individuals to grow longer in terminal length. The nature of that benefit is currently unknown, but suggests that adult terminal length is determined by a factor other than food availability.

Neither adults nor juveniles are randomly distributed among the substrates. One reason for this non-random distribution is that the vegetated rubble substrate might contain the most resources, attracting more individuals. However, there are more adults than juveniles in vegetated rubble and more juveniles than adults in sandy rubble. Perhaps there is enough competition for food resources in the vegetated rubble that juvenile individuals get pushed out to the close-by sandy rubble, a dynamic which follows the theory of ideal free distribution states that it might be more beneficial for individuals to be in a low-density resource poor area than a high-density resource rich area (Ayres pers. comm.).

As a species whose population has been extremely depleted due to harvesting, *L. gigas* needs to be protected from destructive human impact to ensure its survival. Although we
collected all our data in an area labeled as a “replenishment zone” where harvesting is not allowed, 19% of all the shells we found had been harvested. Additionally, 30% of all shells were dead but not harvested. These shells may have moved due to currents or human displacement since harvesting. However, such a high proportion of harvesting is somewhat alarming. These high harvest proportions in the two substrates with the highest abundance of L. gigas suggests that conservation efforts in this area should be focused on creating protection for substrates of coral rubble, both vegetated and sandy. However, the two coral rubble substrates were closest to the line between the replenishment zone and open harvesting. The higher proportion of harvesting in those two substrates could be at least partially due to the proximity of the harvest zone. Given the increased gain from harvesting an adult instead of a juvenile, it is logical that 74% of harvested individuals were adult L. gigas. Unfortunately, the death of one reproductive adult impacts the population significantly more than that of a juvenile due to adult reproductive potential. This therefore furthers the need to protect areas with the highest abundance of adult individuals such as the coral rubble substrates. As found in Clemens-Sewall et al. (2015), MPAs where all fishing is prohibited provide the most effective protection for L. gigas, allowing individuals to grow the largest. While ceasing harvesting of all L. gigas would be most beneficial, it is more realistic to put protections on certain substrates and smaller areas.

Future research should consider substrate preference during reproduction. Studying habitat preference during reproduction could provide a better understanding of seasonal movement of L. gigas and determine if preference shifts during times of reproduction. Additionally, we would like to see how coastal land features impact habitat preference and abundance of L. gigas. For example, we completed our coral rubble (both vegetated and sandy) transects in an area between Owen Island and the reef, features that could be creating a more sheltered environment from larger predators. It would be interesting to test different coral rubble sites around Little Cayman Island to see if there is more variation due to coastal geology than due to substrate type. Additional research could also look at the potential movement of empty shells to understand if harvested or dead shells are moved by currents or other animals. This would allow us to come to stronger conclusions about where individuals are harvested and if including empty shells reflects accurate population numbers across substrates. An additional challenge to determining abundance is that species which aggregate, such as L. gigas, create a spatial heterogeneity that is not often described in surveys of abundance. To properly manage L. gigas, surveys will have to address habitat preference and aggregation of the species across substrates to gain a correct understanding of the population.

Recent studies have shown a decline in L. gigas numbers on the southern side of Little Cayman, which is concerning since a significant area falls into the “replenishment” zone where harvesting is illegal (Bio FSP 2017). The differences across substrates indicate that specific areas, like coral rubble, may be more critical to the population than others. These results emphasize the importance of protecting not only all areas where L. gigas individuals might be found, but also focusing on key areas of vegetated coral rubble substrate. While some species benefit most from protection during their reproductive events, other species, like L. gigas, may benefit most from protection of habitats with highest food resources. Substrate preference might serve as an indicator of vital habitat and provide guidance for conservation efforts hoping to most effectively protect a species.

ACKNOWLEDGEMENTS
We would like to thank Dr. Celia Chen and our TAs for their guidance and insight throughout our project. We would also like to thank Greg Locher for letting us borrow his two kayaks to help with our data collection by Owen Island. Finally, we would like to thank the staff at CCMI for their hospitality.

AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED
REMEMBER THE CHITONS: RESPONSE TO ABIOTIC ENVIRONMENTAL FACTORS IN WEST INDIAN FUZZY CHITON (ACANTHOPLEURA GRANULATA)

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Faculty Editor: Celia Y. Chen

Abstract: Thermoregulation is vital for many organisms. Whether they regulate behaviorally or internally, all animals have methods to adjust their temperature. In the intertidal zone, temperature changes drastically both spatially and temporally. One species that may adjust its behavior to thermoregulate is Acanthopleura granulata (West Indian fuzzy chiton). In this study, we investigated how A. granulata distribution and microhabitat preference are affected by abiotic environmental factors such as light and submersion in water. On Little Cayman Island, we surveyed transects at multiple sites with differing substrates for distributions of A. granulata and the percent coverage of sun. In our experimental setup, we used two tide pools and placed five A. granulata in each of four treatments: sun/submerged, sun/not submerged, shade/submerged, and shade/not submerged (for a total of 40 A. granulata). We then observed throughout the day how the number of A. granulata in each treatment changed. Our results indicate that A. granulata tended to prefer the shade to the sun, and preferred being submerged to being above water. These findings suggest that A. granulata behaviorally thermoregulate. Behavioral thermoregulation is one of many responses to a variety of environmental stressors faced by A. granulata and other organisms in the intertidal zone.

Key words: intertidal, thermoregulation, microhabitat, mollusk

INTRODUCTION
Organisms living in the dynamic intertidal zone must adjust to their constantly changing biotic and abiotic environment. Temporal changes in distribution between microhabitats are caused by both biotic and abiotic environmental changes. Food availability, intraspecific competition, and predation avoidance are all factors that can influence the movement and distribution of organisms in the intertidal zone. For example, one species of gastropod (Nerita atramentosa) adjusts foraging strategies based on shifts in intraspecific competition and in microalgae density and abundance (Chapperon and Seuront 2013). Abiotic factors like the presence and absence of water and sunlight within a habitat can also influence an organism’s movement and distribution (Gerwing et al. 2016).

The changing environmental conditions of the intertidal zone can lead to shifts in the thermoregulation strategies of organisms. For example, a study by Chapperon and Seuront (2013) has shown that numerous species of gastropods living in the rocky intertidal zone exhibit temporal shifts in microhabitat occupation and overall movement in response to temperature variation.

Chiton distribution across different microhabitats (e.g. sun versus shade) demonstrates how microhabitat preference varies as the associated benefits change. Gastropods, specifically chiton, exhibit vertical migration throughout the day in response to changes in tidal levels in order to avoid heat stress and desiccation (Garrity 1984). Poor habitat can increase mortality due to low water exposure, dislodgement, desiccation, and predation (Glynn 1970). One species of chiton, the West Indian fuzzy chiton (Acanthopleura granulata), are found from tide pools to other marine habitats as deep as 780 m (Duperron 2013) and typically feed on a micro- and macro-algal biofilm that is found both in water and on moist surfaces (Dethier and Duggins 1984). Acanthopleura granulata are known to respond to biotic environmental factors: they behave optimally while foraging and moving to avoid predation (Conners et al. 2012). However, it is unclear whether A. granulata optimize their distribution and movement to change abiotic microhabitat conditions for thermoregulatory purposes.

Our study aims to elucidate how A. granulata distribution and microhabitat preference are affected by abiotic environmental factors such as
light and submersion in water. If *A. granulata* does display a microhabitat preference, the proportion of individuals in the sun and shade will remain constant throughout the day despite changes in the proportion and the placement of sun and shade. However, if *A. granulata* does not exhibit microhabitat preference, we would expect the proportion of individuals in the sun and shade to differ between the morning and afternoon as the proportions and positions of sun and shade change throughout the day. The heat stress and desiccation risks often associated with microhabitats in direct sunlight might shift *A. granulata* preference towards areas with shade if individuals are not submerged in water. Alternatively, *A. granulata* might prefer microhabitats in the sun when submerged in water because they are ectothermic and behaviorally thermoregulate with vertical migration as environmental conditions change. Lastly, differences in macrohabitat, such as substrate type or water availability, might affect the observed distribution of *A. granulata*. If *A. granulata* are found in an area with nearly constant water flow, we would expect them to be more evenly distributed between sun and shade than *A. granulata* found in areas with lower water availability.

**METHODS**

We collected data on *A. granulata* distribution and behavior from 1 to 3 March 2017 at Salt Rock, Cumber’s Cove, and a beach between Cumber’s Cove and Salt Rock (referred to as Beach X), Little Cayman Island. Salt Rock is comprised of limestone tide pools, Cumber’s Cove is a beach composed of coral rubble with some large rocky outcroppings, and Beach X is a sandy beach with some small rocky outcroppings. Each location was considered as a different habitat type.

We conducted surveys of *A. granulata* distribution and proportion of habitat availability in sun versus shade at Salt Rock tide pools. At Salt Rock, we surveyed four 20 m transects with 0.25 m² quadrats placed along the length of the transect to quantify sun and shade for the total area. Transects were all equidistant from the water and as close to the tidal splash zone as possible. These transects were surveyed once in the morning and once in the afternoon to account for differences in the distribution of sun and shade as the sun changed position throughout the day. We surveyed one eight meter transect in the morning at Beach X and one eight meter transect in the afternoon at Cumber’s Cove. At all transects, we also counted the number of *A. granulata* within our 0.25 m² plots and whether they were found in the sun or shade.

To analyze *A. granulata* preference, we found 40 *A. granulata* individuals to use as study subjects in four treatments within two separate tide pools at Salt Rock. We observed five *A. granulata* individuals in four different treatments in each tide pool: shaded and submerged in water, sun and submerged in water, shaded and out of water (dry), and sun and dry. We then recorded how the distribution of the 20 individuals changed within each of the tide pools every 60 minutes for eight hours. Partially submerged individuals were recorded as submerged.

To understand the relationship of sun versus shade to the distribution of *A. granulata*, we conducted a chi-square test for each location (Salt Rock, Cumber’s Cove and Beach X) using Microsoft Excel.

**RESULTS**

While we did not observe any *A. granulata* in habitats other than rocky outcroppings that provided opportunities for vertical movement within the splash zone, *A. granulata* were significantly more likely to be in the shade than in the sun. This was measured by the proportion of

<table>
<thead>
<tr>
<th></th>
<th>Salt Rock AM</th>
<th>Salt Rock PM</th>
<th>Cumber’s Cove</th>
<th>Beach 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sun</td>
<td>Shade</td>
<td>Sun</td>
<td>Shade</td>
</tr>
<tr>
<td>Proportion of <em>A.</em> granulata</td>
<td>0.42</td>
<td>0.58</td>
<td>0.41</td>
<td>0.59</td>
</tr>
<tr>
<td>Proportion of quadrat</td>
<td>0.79</td>
<td>0.21</td>
<td>0.73</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 1. Proportions of *A. granulata* found in the sun and the shade compared to the proportion of the environment that is shaded.
A. granulata individuals in the shade relative to the total amount of shade in the environment (Table 1). This was true across habitat types: limestone tide pools at Salt Rock, and sandy beach environments at Beach X and Cumber’s Cove (chi-square = 41.10, $P < 0.001$, df = 1; chi-square = 19.24, $P < 0.001$, df = 1, respectively). Acanthopleura granulata were also significantly more likely to be in the shade throughout the day, as shown by the results from the morning and the afternoon at Salt Rock (morning: chi-square = 114.3, $P < 0.001$, df = 1; afternoon: chi-square = 108.1, $P < 0.001$, df = 1). Sun and shade proportions were similar at both times of day (79% sun in morning, 73% sun in afternoon; Table 1).

DISCUSSION

Acanthopleura granulata were significantly more likely to be in the shade than in the sun, despite the relatively higher availability of habitats in the sun. The fact that this trend was the same regardless of time of day (Salt Rock AM and PM) suggests some sort of behavioral thermoregulation by A. granulata. Even though the proportion of the environment that was shaded remained mostly constant throughout the day, the changing position of the sun caused different areas to be shaded in the afternoon. Therefore, without some degree of

![Figure 1. Distribution of A. granulata in both tide pools across treatments at each observation time.](image)

In the experimental treatments, we observed that at midday, the highest number of A. granulata were in the sun. The number of A. granulata in the water also peaked at this time. By the end of the day, the shaded and submerged treatment had the most individuals (Fig. 1). Our field experiments also showed an overall preference for shade. Over the course of the day, the largest change was a move into the shaded and submerged treatment (Fig. 2). The lowest number of A. granulata were in the sun and dry treatment.

![Figure 2. Change in the number of A. granulata in each treatment over the whole observation period (09:40-16:40). Each treatment started with five A. granulata at 09:40.](image)
indicate that A. granulata prefer shaded microhabitats in the intertidal zone.

The seeming preference for shaded habitats was also consistent across habitat types. If the shade-heavy A. granulata distribution was specific to the Salt Rock limestone tide pools, then the trend might have been site-specific, but the same result was seen at both Cumber’s Cove and Beach X, each of which are distinctly different habitats. Cumber’s Cove and Beach X both had more horizontal surfaces (rock outcroppings and coral rubble within a sandy beach) than Salt Rock, yet had similar proportions of sun versus shade. Beach X had more sun overall, but the distribution of A. granulata still followed the same trend toward shade as was found at Salt Rock. This similarity across macrohabitat sites provides further evidence that A. granulata display microhabitat preferences.

The observation that the number of A. granulata in the sun peaks at midday is consistent with that time of day when the sun exposed areas are the highest and shade is most limited. However, it is interesting that this is paired with an increase of individuals submerged and in the sun that peaks at the same time. Being in the water may be more important than either sun or shade overall, as it would be better to be in the sun and wet than in the shade and dry. On the other hand, leaving the water might allow A. granulata to escape marine predators as predation has been shown to increase during high tide when more individuals are submerged (Carter et al. 2012).

Our findings suggest that A. granulata regulate their temperature behaviorally in the intertidal zone. This makes sense due to the need to retain moisture to avoid desiccation and because in water they are exposed to a smaller range of temperatures than in air, which could help individuals maintain an even temperature. Although A. granulata can hold water in their shells, they often lift their shells to breathe so are constantly exposed to their surroundings (UCSB 2011). Another benefit of staying in the water is that biofilm, the main food source of A. granulata, grows underwater and in wet areas (Dethier and Duggins 1984). The observed A. granulata preference for shade might be because it provides a habitat with a more consistent temperature than in direct sunlight. Acanthopleura granulata might experience several environmental and biological stressors, including intraspecific competition, limited food availability, and predation by sea stars, crabs, fish, anemones, and sometimes sea birds (MESA 2015). A study by Chapperon and Seuront (2013) on shifts in microhabitat in an intertidal gastropod (Nerita atramentosa) found that microhabitat occupation and aggregation are a response to a combination of resource distribution, temperature, desiccation, and wave action. Similarly, A. granulata distribution across different microhabitats in the intertidal zone may be a result of this combination of stressors, including wave action that abrades shells, constant predation pressure, and biofilms releasing products that can lower pH around the shell (Sigwart et al. 2015). Although our study only considered the effects of temperature and desiccation on A. granulata microhabitat distribution, future studies might examine the effects of food availability, intraspecific competition, and predation avoidance on chiton distribution.

In the future, studies to better understand the seeming preference of A. granulata for vertical surfaces could compare distribution and abundance across vertical and horizontal habitats. There is the potential that vertical faces allow individuals to move between submerged and not-submerged locations more efficiently, easing processes of behavioral thermoregulation rather than having to travel farther distances to leave or enter water on horizontal surfaces. Additionally, as A. granulata in this study were found on textured rock surfaces, a study of habitat rugosity could further explain A. granulata distribution. More transects taken at regular intervals throughout the day would provide more information about change in sun and shade proportions in the environment over the course of the day and could also allow for a better understanding of A. granulata distribution over time. We would also like to investigate rate of movement of individuals and track movement over the course of a day in relation to the movement of shade and sun in the environment. An experiment of shading individuals and tracking their movement might provide better understanding of individual preference and behavior. Finally, as A. granulata and other chiton species range from intertidal zones to deeper than 780 m, habitat preference and thermoregulatory behavior likely varies across depth. A study testing habitat preference and thermoregulation across...
habitat depths could further demonstrate the driving factors behind chiton distribution.

_Acanthopleura granulata_ appear to adjust to abiotic conditions in their environment, such as changes in sun exposure and water level, by behaviorally thermoregulating. Thermoregulation is one of the challenges that affect _A. granulata_ and other organisms living in the intertidal zone. These organisms may be exposed to environmental stressors ranging from wave action to constant predation pressure. Optimizing a balance between the costs and benefits of living in each band of the intertidal zone is essential for survival in such a dynamic environment.

**LITERATURE CITED**


IT TAKES GUTS TO COMBAT INVASIVE *Pterois volitans* AT LITTLE CAYMAN

AMBER AHRONIAN, LOUISE BARIAS, REBECCA FLOWERS, AND EMILY OKUN

Faculty Editor: Celia Y. Chen

Abstract: Lionfish (*Pterois volitans*) is a highly invasive species that affects the structure of coral reef communities in Atlantic marine ecosystems because of their high predation pressure on native organisms. Since their introduction to Little Cayman in 2008, culls have targeted *P. volitans*, but little is known of their reproductive physiology and diet. Previous studies have shown that male *P. volitans* have greater body fat than females. We investigated whether this was due to differences in reproductive investment and diet. If males and females differ in percentage of body fat because they are investing more energy into reproduction, we would expect females to have a greater gonadosomatic index (GSI) than males. If males have lower GSI and are investing in growth instead of reproduction, we would expect males to be longer than females. Finally, if there is a difference in diet composition between the sexes due to their differences in energy investment, we would expect that females eat more vertebrates for their higher energy density. We calculated the reproductive investment, percent body fat and diet composition of 40 lionfish. We found that females have a significantly lower percentage of body fat and higher reproductive investment than males. We also found that males were longer than females, suggesting that males invest more in growth than in reproduction. However, diet composition between male and female lionfish was similar. Our results indicate that, while male and female lionfish are consuming the same prey, they are allocating their consumed energy differently. Our findings that females invest a large amount of their energy into reproduction can inform future modelling and management of population growth.

Key words: diet, GSI, invasive, lionfish, *Pterois volitans*, reproductive investment, stomach content analysis

INTRODUCTION

The number of invasive species worldwide has increased by orders of magnitude in the past 200 years (Mack et al. 2000). Many invasive animal species can consume disproportionately large amounts of native prey, but it is unclear how they are allocating this extra energy. They may be investing in reproduction or growth. Studying the physiology of the invaders can help to understand how individuals of the current population are contributing to population growth in the future, and can therefore inform conservation efforts of native prey species.

Lionfish (*Pterois volitans*) are well-known invaders of Atlantic marine habitats, where they are prolific predators and cause devastating effects on reef fish diversity and abundance. Release from predation and their ability to consume prey of up to half their body length allows *P. volitans* to grow 1.25-2.25 times faster in the Atlantic than in their native range in the Pacific (Pusack et al. 2016). The huge amount of prey they consume can lead to drastic changes in native prey populations.

Green and colleagues (2012) showed that their introduction coincided with a 65 percent decline in biomass for 42 Atlantic prey fishes over just two years. As such, *P. volitans* is an ideal invasive animal species in which to study physiology and energy allocation strategies because there is urgent need to understand the mechanisms of their impact and create effective management strategies.

*P. volitans* was first observed in Little Cayman in 2008. Locals have implemented weekly culls to keep their population low. However, the exact impact of *P. volitans* on the local fish community, and the exact benefits *P. volitans* are reaping from the local population, remain poorly understood here, as they do elsewhere (Edwards et al. 2014). Studies in nearby areas of the Caribbean have shown contradictory results as to how much of the *P. volitans* diet is made up of vertebrates in comparison to invertebrates. Muñoz et al. (2011) found that the diet of *P. volitans* in the Caribbean was 99 percent fish, while Morris et al. (2009) found that their diets consist of 78 percent teleosts and 14 percent crustaceans. Clearly, *P. volitans* in the Caribbean are benefitting from feeding on native populations, but it is unclear which prey types are most affected and how *P. volitans* are investing energy obtained from these prey.

Female *P. volitans* have a lower percentage of body fat than males (Costello et al. 2012). This raises the question, is the difference in percentage...
of body fat due to reproductive investment or diet composition? If reproductive investment is different between the sexes, we would expect that females invest more in reproduction than males. This would result in a higher gonadosomatic index (GSI) and lower percentage of body fat in females. Then, if males have lower reproductive investment, and a higher percentage of body fat, males might instead be investing energy in growth, which would aid in territory defense. We would then expect males to be larger than females. Finally, if this difference in energy investment exists between the sexes, there might also be a difference in diet composition. Females may have to consume prey at higher trophic levels with greater energy content, and would therefore consume more vertebrates. For males, energy-dense food would be less necessary because they have less reproductive investment, resulting in a diet higher in invertebrates, which might be easier to catch but have a lower energy content. Alternatively, males and females may take in the same amount of energy, but invest it differently, in which case there would be no difference in diet composition.

METHODS
We measured and weighed 18 female and 22 male *P. volitans* collected on two culls around Little Cayman on 1-2 March 2017. We then dissected the internal organs of the fish in order to measure the mass of the gonads, fat stores, and stomach content of each fish. To do so, we: 1) removed and weighed the ovaries or testes; 2) removed and weighed the fatty tissue deposits that coated the internal organs; 3) dissected the stomach and squeezed the intestinal tract to remove the gut contents. We then visually examined the gut contents and recorded if there was evidence of invertebrates (such as a shrimp head or a claw) or vertebrates (such as scales or whole intact fish). From these data we assigned each fish to one of four diet categories based on their prey type(s): 1) evidence of vertebrates only, 2) evidence of invertebrates only, 3) evidence of both vertebrates and invertebrates, or 4) no evidence of prey type. We calculated the GSI of each fish by dividing the mass of an organism’s gonads by its total mass. We calculated the percentage body fat for each fish by dividing the mass of its fat by its total body mass.

We arcsine transformed GSI for all analyses in which it was a response. We conducted an ANOVA to test whether GSI was affected by sex, length, diet, percentage body fat, gut mass, length*sex, sex*percentage body fat, and diet*percentage body fat. We performed linear regressions to test for an effect of total mass on
gonadal mass for each sex. We conducted another ANOVA to test whether percentage of body fat was affected by sex, length, diet, gut mass, length*sex, length*diet, and diet*sex. We performed two linear regressions to test for an effect of percentage of body fat on GSI for each sex. We used another ANOVA to test whether length was affected by diet, sex, and diet*sex. We also ran a chi-square analysis to determine whether diet composition varied between sexes.

**RESULTS**

Percentage of body fat and reproductive investment were significantly different between sexes. Percentage of body fat by mass was significantly higher in males (mean ± SE = 1.1 ± 0.18%) than females (mean ± SE = 0.39 ± 0.22%), but did not vary significantly with any other variables (Table 1; Figure 1).

| Table 1. ANOVA of fish sex, length, diet, gut mass, and their interactions on percentage of body fat by mass. |
|---|---|---|---|---|
| df | SS | F | P |
| Sex | 1 | 0.000 | 7.49 | 0.01 |
| Length | 1 | 0.000 | 0.34 | 0.57 |
| Diet | 3 | 0.000 | 1.13 | 0.36 |
| Gut mass | 1 | 0.000 | 1.57 | 0.22 |
| Length*sex | 1 | 0.000 | 0.25 | 0.62 |
| Length*diet | 3 | 0.000 | 0.26 | 0.85 |
| Diet*sex | 3 | 0.000 | 1.85 | 0.16 |
| Error | 26 | 0.001 | - | - |
| Totals | 39 | 0.002 | - | - |

GSI was significantly higher in females (mean ± SE = 4.1 ± 1.15%) than males (mean ± SE = 0.00 ± 0.77%) but did not vary significantly with any other variables (Table 2; Figure 2).

Total mass had a significant positive effect on gonadal mass in both sexes, but ovary mass increased more drastically with body mass in females (slope = 0.06 ± 0.03, P = 0.03, r = 0.27) than did testes mass in males (slope = 0.001 ± 0.0003, P = 0.002, r = 0.44). While not significant, there was a trend toward a negative effect of percentage of body fat on GSI in females (slope = -2.22 ± 1.21, P = 0.08, r = 0.17). GSI was not significantly related to percentage of body fat in males (slope = 0.03 ± 0.03, P = 0.38, r = 0.05) (Figure 3).

Mean length was significantly different between sexes. Males were significantly longer (mean ± SE = 296 ± 8.17 mm) than females (mean ± SE = 261 ± 8.34 mm) (F<sub>4,35</sub> = 8.34, P = 0.007).

Diet composition was not significantly different between sexes (chi-square = 1.58, P = 0.66, df = 3; Figure 4). Diet composition also did not vary with length, for males and females (F<sub>4,35</sub> = 1.05, P = 0.39).

**DISCUSSION**

We found that males had a significantly higher percentage of body fat than females, which is consistent with previous studies (Costello et al. 2012). We also found that reproductive investment was significantly greater in females than in males. These results suggest that, as expected, females are investing most of their energy into the production of eggs, and therefore have a smaller amount of fat reserves. In contrast, the production of sperm in males is not as energetically costly as the production of eggs, so males are able to store more fat.

GSI was significantly higher in females (mean ± SE = 4.1 ± 1.15%) than males (mean ± SE = 0.00 ± 0.77%) but did not vary significantly with any other variables (Table 2; Figure 2).

Furthermore, the relationship between percent body fat and reproductive investment was not significant in male *P. volitans*. This may be because we measured GSI in males as the mean of each individual's testes masses, which only varied from 0.1 to 1.0 grams, whereas a measure of sperm count may have more information about male reproductive investment. Yet, since males do not have to invest much energy into the production of sperm and testes, it follows that the percent body fat is independent of their reproductive investment. On the other hand, we did observe a
trend between lower percentage of body fat and greater reproductive investment in females. This again suggests that females are investing more of their energy into their reproduction. To determine if there is a significant trend, we would need a larger sample size of female *P. volitans*.

We also found that males are significantly longer than female lionfish. This is supported by their life history, as males are territorial, and their efficacy in territoriality is probably helped by a larger size. Thus, dimorphism in body fat might be the result of investing in reproductive fitness through different avenues: females investing in gamete production and males investing in growth for territoriality.

We did not find differences in the diet composition of male and female lionfish. Diet did not vary across lionfish sizes; larger lionfish were eating about as many vertebrates as smaller lionfish. These results suggest that the differences observed in body fat between males and females are most likely explained by their differences in reproductive investment, but not by their diet.

However, these results could be due to limits in our methodology. We determined individual *P. volitans* diets based on a stomach content analysis, which provides only a snapshot in time of the food the fish consumed right before it was harvested. In the future, observational field data could give a more complete view of how frequently lionfish consume vertebrate versus invertebrate prey items. Observational studies would also be more conducive to identifying prey down to genus or species level, which would allow for energy density or lipid content analysis of the different prey taxa.

The pattern of energy allocation in male and female *P. volitans* has important implications for their management. Similar studies have investigated the physiology of other invasive species. Joanna and colleagues (2011) found growth rate differences between males and female amur sleepers (an invasive fish species in Europe), are likely due to unequal reproductive investment. The authors suggested that it may contribute to invasive success of the fish, as they are able to reproduce more quickly. This may also be the case in *P. volitans*. Female *P. volitans* invest more heavily in reproduction than males, so culls targeting females may reduce population growth. However, the culls from which we obtained our samples contained a nearly even proportion of both sexes. We believe these non-targeted culls are the most effective strategy because both sexes consume large amounts of native prey populations, and the sexes are difficult to distinguish in the wild.

*P. volitans* size, rather than sex, may be a more important determinant of individual impact. Large *P. volitans* consume larger prey and have larger gonads, which means that they have higher
reproductive potential than smaller *P. volitans* individuals. As such, current management efforts, which target large fish of both sexes, are likely effective in controlling population growth and predation pressure of the invasion *P. volitans*. Frazer et al. (2012) found that culls in Little Cayman have led to a reduction in population density and mean size of *P. volitans*.

An understanding of the energy allocation specific to a particular invasive species is important to inform management practices. The tradeoffs between reproductive investment and growth are particular to a species and of often groups, such as sexes, within a species. As such, physiological information is necessary to ensure that management practices, including culls, are achieving their intended goals.

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AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED


AN INVESTIGATION OF DIEL MIGRATION AND FEEDING ACTIVITY IN CARIBBEAN POPULATIONS OF SILVERSIDES, *MENIDIA* SPP.

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Abstract: Predatory behavior is often coupled with the characteristic behaviors of prey. Our study investigates how predator-prey dynamics affect the diel patterns of *Menidia* spp. (silversides) and their zooplankton prey. *Menidia* range from Nova Scotia, Canada to the Caribbean and display plastic diel migratory patterns that vary by population. Zooplankton (*Menidia*'s primary prey) are often most abundant in deeper, offshore waters during the day and move upward in the water column and towards shore at night, though this pattern has not been as thoroughly investigated in shallower reef systems. We hypothesized that *Menidia* abundance in Central Caribbean back reefs corresponds to the diel vertical migrations of zooplankton. Through field surveys, we examined how time of day affects the abundance of *Menidia*, density of zooplankton, and habitat temperature. The mean fish and zooplankton abundances were significantly greater during the night. The water temperature fluctuated more than 4°C between daytime and nighttime. Our study suggests that zooplankton in shallow reefs display diel vertical migration patterns and that *Menidia* exhibit migratory behavior corresponding to the movement of zooplankton. The daily temporal variation in water temperature might play a larger role in diel migrations of *Menidia* and its zooplankton prey than expected, potentially through effects on metabolic rate. Such a discovery may offer additional insight into diel migratory patterns of other planktivorous fish, especially in those species with ranges extending across temperate and tropical regions.

Key words: diel migration, *Menidia*, zooplankton

INTRODUCTION

Predator-prey relationships direct organisms’ continual adaptations as they engage in evolutionary arms races for survival. As prey evolve strategies to evade predators, predators continually evolve strategies to better capture their prey. Over time, these relationships could lead to predictable patterns of predator and prey behaviors that can be used to enhance our understanding of one or both organisms. Such patterns are evident in the diel migrations of marine organisms (Berge et al. 2009), which might evolve to manipulate the temporal and spatial relationships between predator and prey species.

*Menidia* range from Nova Scotia, Canada to the Caribbean and often inhabit estuaries and lagoons (Conover 1992, Bengtson 1981). Like many fish species, they display distinct daily patterns in migratory behavior (Humann and Deloach 1989, Middaugh 1981). Previous research has indicated that *Menidia menidia* are often more abundant in estuaries during the night, although these results are not universal (Rountree and Able 1993). For example, Reis and Dean (1981) observed greater abundances of *M. menidia* in South Carolina during the day than during the night. There is precedent for such plasticity in diel pattern within species, as different populations of *M. menidia* have exhibited either diurnal or nocturnal behaviors (Reebs 2002). There are several potential explanations for differences in diel patterns in *Menidia*, including predator avoidance, reproductive, and hunting behaviors (Rountree and Able 1993). It is also possible that variation in temperature may account for diel behavior in *Menidia*, although this may be unlikely in Little Cayman reef ecosystems because these reefs are documented to thrive in high levels of climatic stability, often varying just 0.5°C (28.0-28.5°C at 1.2m depth) throughout the day (Zhu et al. 2014).

Zooplankton, *Menidia*'s primary prey, also behave in distinct patterns throughout the day (Gilmurray and Daborn 1981, Reinfelder and Fisher 1994). In deep fringing reefs, zooplankton perform diel vertical migrations such that they are most abundant in deeper, offshore waters during the day and move upward towards shore at night (Gilmurray and Daborn 1981, Reinfelder and Fisher 1994, White 1998). This pattern is often attributed to the trade-off between food acquisition and predator avoidance. Zooplankton feed on...
phytoplankton, photosynthetic organisms that occur in greater abundances where light is available near the surface of the ocean (Berge et al. 2009). Thus, being near the water’s surface puts zooplankton in proximity to their primary energy source (Berge et al. 2009). However, higher light availability also causes zooplankton to be more visible, and thus more vulnerable to predators, many of which are visual hunters (Berge et al. 2009). Therefore, zooplankton in deep fringing reefs might display diel vertical migration patterns so they can feed on phytoplankton near the surface at night, when visual predation is minimized, and evade predators in deeper, darker waters during the day (Berge et al. 2009).

However, the diel patterning observed in zooplankton of offshore waters has not been widely documented in shallow reef environments. Like in offshore systems, there is evidence that zooplankton abundance increases in shallow reefs at night. Yet, daytime zooplankton abundances are often lower in reef shallows than in deep reefs (Sorokin and Sorokin 2009, Sorokin and Sorokin 2010). Furthermore, evidence suggests that zooplankton diel behavior differs in shallow and deep reefs. Alldredge and King (2009) found that zooplankton do not display significant diel patterns in shallow reefs, although they are more abundant near the water’s surface. These results suggest that zooplankton in shallow reefs might not seek deeper waters for predator avoidance, as they might be ubiquitously attracted to light (Alldredge and King 2009). Thus, further evidence is needed to elucidate whether the previously described explanation for diel vertical migration in deep reefs can be extended to shallow reef lagoons.

If *Menidia*’s zooplankton prey perform daily vertical migrations, this might affect *Menidia*’s daily behavioral trade-offs between food acquisition and predator avoidance, as moderated by physical environment. *Menidia* might have an advantage if they feed during the night such that they coincide spatially and temporally with their zooplankton prey when zooplankton densities are highest. By adapting to follow the diel migrations of zooplankton, planktivorous fish could gain access to a vulnerable food source. This potential advantage could produce pressure selecting for a cascade of behavioral patterning based on trophic interactions. Our study elucidates whether *Menidia* found off the coast of Little Cayman display nocturnal diel patterns and whether their activity is influenced by the daily vertical migration of zooplankton prey. We hypothesize that *Menidia* local abundance corresponds to the diel vertical migrations of zooplankton. We predict that both *Menidia* and their zooplankton prey will exhibit higher shoreline abundances at night. Alternatively, *Menidia* abundance might not be correlated with diel zooplankton migrations, but rather might respond to other environmental conditions.

**METHODS**

*Field and Lab*

Through field surveys, we examined how time of day effects habitat temperature, abundance of *Menidia*, and density of zooplankton. On the 2nd and 3rd of March 2017, we set three 40 meter transects separated by 10 meters parallel to the shore at Grape Tree Bay in Little Cayman. Transects were anywhere from 5 meters to 25 meters away from the shore, and the substrate varied from turtle grass to sand. Maximum depth of collection was approximately 1.5m. We collected fish from 14:00 to 16:00 to assess daytime populations and from 20:00 to 22:00 to assess nighttime populations. To collect fish, we walked along the transect, passing a net through all levels of the water column every 5 meters. This netting method was designed to capture *Menidia* in both the water column and near the benthos, such as in turtle grass. We visually inspected the water to ensure that our results adequately represented the population. At night, because flashlights attract *Menidia*, visual assays were not conducted. We also conducted plankton tows along the same transects using a 30 cm tow net. Plankton were removed by using seawater to rinse the contents of the net into bottles. We placed a HOBO temperature logger in the lagoon where fish were collected to record temperature throughout the length of our study.

In the lab, we filtered out the zooplankton from each transect and then concentrated each solution to a volume of 100 mL. We thoroughly mixed each solution to ensure zooplankton were evenly distributed, then pipetted three 10 mL subsamples, which we viewed under a dissecting microscope to determine the abundance and broad
taxa of zooplankton. We multiplied the summed zooplankton abundances from the subsamples for each transect by 10/3 to estimate the total zooplankton in the transect sample. We then calculated the density of zooplankton by dividing the zooplankton count by the cylindrical volume towed by the net along the 40 meter transect.

**Data Analyses**
We performed two Mann-Whitney U tests to determine the effect of time of day on zooplankton density and *Menidia* abundances. We plotted temperature through time from 16:00 on 2 March 2017 until 00:00 on 3 March 2017.

**RESULTS**

**Observational Results**
When collecting at night, we noticed that larger *Menidia* appeared in transects at greater depths in the lagoon, towards the reef. While we were able to sweep for fish within the entirety of the water column at each 5 m transect interval at shallower depths, we were often unable to comprehensively sweep the deeper areas of transects closer to the reef, which may explain the decreases in fish abundance. Although we did not collect any *Menidia* in our daytime transects, we did observe a few small schools close to shore.

![Figure 1](image1.png) **Figure 1.** The abundance of *Menidia* was higher during the night than during the day.

![Figure 2](image2.png) **Figure 2.** The density of zooplankton was higher during the night than during the day.

![Figure 3](image3.png) **Figure 3.** *In situ* water temperature through our testing period. Arrows indicate when animals were sampled; shaded areas indicate night.
Data Analyses

The mean fish abundance was significantly greater during the night (10.67 ± 4.67) than during the day (mean = 0.00 ± 0.00) (chi-square = 4.35, P = 0.04, df = 1; Fig. 1). The mean zooplankton density (individuals/mL) was also significantly greater during the night (48.53 ± 10.95) than during the day (5.01 ± 0.78) (chi-square = 3.84, P = 0.0495, df = 1; Fig. 2). Common zooplankton belonged to the orders Amphipoda, Euphausiacea, Decapoda, Mysidacea, and Ostracoda and the subclass Copepoda. During our test period, temperature was lowest (26.9°C) around 06:00 and highest (31.0°C) around 16:00 on 3 March 2017 (Fig. 3). The water was 29.1°C when sampling *Menidia* at 16:00 on 2 March, 28.0°C when sampling *Menidia* and zooplankton at 21:00 on 2 March, and 30.9°C when sampling zooplankton at 15:00 on 3 March.

Discussion

Our results provide evidence that *Menidia*’s nocturnal activity may have emerged to increase their temporal and spatial proximity to their zooplankton prey. The data indicate that the abundance of *Menidia* and the density of zooplankton are significantly higher during the night than during the day. The observed diel patterning of zooplankton abundance in this lagoon system supports that the diel vertical migrations found in offshore zooplankton species may indeed apply to shallower back reef environments. Our discovery of small schools of *Menidia* close to shore during the day suggests that populations might rest directly along the shoreline during the daytime, albeit in significantly lower abundances than at night. In conjunction with our measures of *Menidia* abundance, a significant increase in zooplankton density at night supports the hypothesis that *Menidia* abundance correlates with zooplankton density. However, since the few schools of *Menidia* observed during the day appeared to congregate in the same depth as those *Menidia* collected at night, *Menidia* does not appear to follow vertical migratory pattern parallel to that of the zooplankton. Rather, it appears that *Menidia* might migrate from the shoreline or from other areas near the surface into the lagoon at night as they converge with rising zooplankton populations. Regardless, spatial and temporal synchronization of diel abundances of *Menidia* and their zooplankton prey could suggest that prey availability plays a role in diel patterning of feeding behavior in *Menidia*.

Temperature might also play a significant role in the diel migrations of *Menidia*. Daily water temperature measurements fluctuated by up to 4°C throughout the day (26.9-31.0°C). This variation in water temperature is dramatically greater than the variation recorded in literature on Little Cayman reefs (0.5°C variation recorded at 1.2 m, Zhu et al. 2014). Such an increase from literature values could be because our collection occurred within a shallow lagoon that might be more susceptible to greater daily heating and lower water circulation than water closer to the reef itself (Zhu et al. 2014). It is likely that these temperature variations have physiological consequences for *Menidia*. In marine ectotherms, temperature directly affects physiological performance, with increases in temperature causing disproportionate decreases in water oxygen concentration due to the nonlinear relationship between temperature and oxygen solubility (Conover 1992). Furthermore, variation in juvenile growth is often considered to be a result of environmental limitations (e.g. temperature and food availability) rather than selection (Billerbeck et al. 2001). Thus, variation in temperature can severely impact metabolic rate and consequently can impact feeding patterns of *Menidia* throughout the day. Previous studies have found that the maximum prolonged and burst swimming speeds of *Menidia* from higher latitudes (Nova Scotia) were significantly lower than those of populations from lower latitudes (South Carolina) (Billerbeck et al. 2001). Moreover, maximal growth rates of larval and juvenile *M. menidia* are 1.5-3.0 times higher in populations from northern latitudes than those from southern latitudes, depending on temperature (Present and Conover 1992, Billerbeck et al. 2000). Thus, there is evidence that differences in water temperature affect the physiology of *Menidia* species across their latitudinal ranges.

Since the Caribbean appears to be the southern extreme of reported *Menidia*, higher ocean temperatures in the tropics could serve as a limiting factor in *Menidia* feeding and metabolism. Previous studies of *M. menidia* have established 23-25°C as an intermediate temperature range for *M. menidia* distribution.
across latitudes, with 28°C set as an upper thermal limit for examining metabolism and swimming (Billerbeck et al. 2001). Higher metabolic rates due to higher ocean temperatures during the day (e.g., as high as 31°C in this system) could prevent Menidia from expending additional energy towards foraging during the day. On the other hand, lower water temperatures at night could enable a lower ectothermic metabolic rate, allowing Menidia to devote less energy towards metabolism and more energy towards foraging. Along with its potential benefits for predation avoidance, the diel vertical migration of Menidia’s zooplankton prey may also involve a response to temporal temperature change. Previous studies off of North Carolina have found that diel vertical migrations in zooplankton track vertical changes in oceanic thermoclines, with populations migrating to deeper waters during the day as summer ocean temperatures increase (Marcogliese and Esch 1992). Vertical migration might afford zooplankton a metabolic advantage by reducing energy expenditure during the day (Marcogliese and Esch 1992). Our study system might mirror these offshore findings within back reef systems, with drastic increases in temperature at the surface of the shallow lagoon during the day discouraging foraging in both zooplankton and Menidia alike. Although our data suggest a correlation between Menidia abundance and zooplankton density throughout the day, further studies are needed to provide sufficient evidence for causation. Given our limitations in time and resources, we were unable to simulate Menidia foraging across different temporal and lighting conditions in the laboratory. We were able to successfully hold live Menidia in captivity, as well as collect mysid shrimp (order Mysidacea) for measuring foraging rate. However, the mysid shrimp perished quickly in captivity, and the Menidia did not feed on the mysids or any other food we provided, most likely due to high stress from captivity. We encourage investigations with a greater ability to maintain and successfully feed Menidia populations to examine the effects of circadian rhythms and light exposure on the diel feeding and activity patterns of Menidia. Furthermore, studying the effect of temperature, light, and time of day on Menidia across its latitudinal range of the Atlantic could reveal further differences in feeding and migratory behavior among populations. Although our plankton tows seemed to adequately represent zooplankton density during both day and night, a more comprehensive netting strategy, such as by seine, could better represent Menidia abundances at night, especially at depth.

Our study suggests that Menidia in the Central Caribbean exhibit migratory behavior that is correlated with the diel vertical migrations of its zooplankton prey and with temperature. Such a discovery may offer additional insight into potential diel migratory patterns of other planktivorous fish, especially in those species with ranges extending across both temperate and tropical regions. Further examination of the effect of diel migrations on upper trophic levels is warranted. As planktivorous fish species are a critical energy source for marine species of higher trophic levels, it is plausible that predatory fish will change their behavior in response to the behavior of their piscine prey. Menidia’s foraging response to zooplankton migrations might influence the behavior of Menidia’s predators, producing a trophic behavioral cascade. Knowledge of the feeding patterns and distributions of planktivorous fish is critical for understanding the broad influences of the variable spatial-temporal patterns of plankton on the marine food web.

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AUTHOR CONTRIBUTIONS
All authors contributed equally.

LITERATURE CITED


May the stories and studies of the Bio FSP in its 40th year offer guidance to aspiring ecologists for many years to come.