

# PARTICIPANTS OF THE DARTMOUTH BIOLOGY FSP 2018

## **FACULTY**

CELIA CHEN, MATTHEW P. AYRES, MARK LAIDRE

## **LAB COORDINATOR**

CRAIG D. LAYNE

## **GRADUATE ASSISTANTS**

DEBORA GOEDERT, MICHAEL BUTLER BROWN

## **UNDERGRADUATE STUDENTS**

ALEC COBBAN  
ALEXANDER COTNOIR  
ALEXANDRA STENDAHL  
ANGELA ORTLIEB  
BALTHASAR VON  
HOYNINGEN HUENE  
CLAYTON JACQUES

CORINNE VIETORISZ  
GRACE CALLAHAN  
HANNAH HOFFMAN  
MAANAV JALAN  
MARY PEDICINI  
PAUL VICKERS

## **Dartmouth Studies in Tropical Ecology, Vol. 28 (2018)**

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

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

We 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](mailto:Matthew.P.Ayres@dartmouth.edu) and the administrative assistant is Sherry L. Finnemore, [Sherry.L.Finnemore@dartmouth.edu](mailto:Sherry.L.Finnemore@dartmouth.edu).

Matt Ayres  
Hanover NH, USA  
28 Dec 2018



# COSTA RICA 2018 SCHEDULE

Bio FSP 2018. Detailed schedule for Costa Rica. 26 Dec 2017

Date	Day	Location	Morning	Afternoon	Evening
3-Jan	Wed	To San Jose	Travel	Travel	Arrive in evening
4-Jan		In San Jose	OTS, Program overview	San Jose exploration	Group dinner in SJ
5-Jan		To Palo Verde	Travel to Palo Verde	Orientation. Research tactics 1	<b>Lec: Intro CR ecol (ML,MP)</b> . Nightwalk
6-Jan		At PV	Orientation. <b>Vertebrates (DG)</b> .	Tactics: questions. <b>Arthropods (DG)</b>	Tactics: Hypotheses & predictions.
7-Jan	Sun	At PV	SIP-1 research development	<b>Plant lab (MBB)</b> . SIP-1 proposals	<b>Lec: Primates (ML, AO)</b> .
8-Jan		At PV	SIP-1 pilot studies	Tactics 3: Statistics. SIP-1 research	Tactics: Theories & context.
9-Jan		At PV	SIP-1 research	SIP-1 research	<b>Lec: Social Insects (ML, BvH)</b> . Writing lab.
10-Jan		At PV	Vert practicum. SIP-1 research	SIP-1 research	SIP-1 symposium. SIP-1 v01 due
11-Jan		At PV	River trip	SIP-1 revisions	SIP-1 revisions; nightwalk
12-Jan		To Junquillal	Bus to trailhead. Hike to beach.	Orientation. <b>Sea Turtles (ML, CJ)</b>	Field: Sea turtle nesting
13-Jan		At Junquillal	Orientation. <b>Mangroves (MBB)</b>	Reconnaissance. <b>Hermit crabs (ML)</b>	Field: Sea turtle nesting
14-Jan	Sun	To Monteverde	Travel	Travel. Orientation.	Revisions of SIP-1; SIP-1 v02 due.
15-Jan		At MV	Orientation & reconnaissance	SIP-2 research development	SIP-2 proposals. SIP-1 accepted & filed.
16-Jan		At MV	Hummingbirds. Develop SIP-2s	SIP-2 research	<b>Lec: Avian Ecology (DG, MJ)</b>
17-Jan		At MV	SIP-2 research	SIP-2 research	SIP-2 research
18-Jan		At MV	SIP-2 research	SIP-2 research	<b>Lec: Diversity (MBB, ACot)</b>
19-Jan		At MV	SIP-2 research	SIP-2 research	<b>Discussion: The purpose of science</b>
20-Jan		At MV	Cloud forest recon. Revisions.	SIP 2 analyses & context	SIP-2 symposium. SIP-2 v01 due.
21-Jan	Sun	To Cuerici	Travel to San Jose	Travel to Cuerici; Orientation	<b>Lec: Coevol 1 (MA, PV)</b>
22-Jan		At Cuerici	Orientation & reconnaissance	SIP-3 planning / proposals	Writing. SIP-2 v02 due
23-Jan		At Cuerici	SIP-3 research	SIP-3 research. SIP-2 revisions.	<b>Lec: Coevol 2 (MA)</b>
24-Jan		At Cuerici	SIP-3 research	SIP-3 research	SIP-3 research
25-Jan		At Cuerici	SIP-3 research	SIP-3 analyses & context	SIP-3 symposium
26-Jan		At Cuerici	SIP-3 v01 due	Reconnaissance. Writing. Revisions.	Writing. SIPs 1-2 accepted & filed.
27-Jan		To El Campanario	Hike. Bus to Sierpe.	Boat to El Campanario	<b>Bats &amp; katydids (HH)</b>
28-Jan	Sun	At El Campanario	Orientation & reconnaissance	<b>Eleutheroactyls (RC)</b> . SIP-4 development.	SIP-4 preproposals. Nightwalk
29-Jan		At El Campanario	Orientation	SIP-4 planning and preproposals	<b>Lec: Cons Bio 1 (MBB)</b>
30-Jan		At El Campanario	SIP-4 pilot studies	SIP-4 research	Nightwalk
31-Jan		At El Campanario	SIP-4 research	SIP-4 research	Nightwalk
1-Feb		At El Campanario	SIP-4 research	SIP-4 analyses & context	SIP-4 symposium.
2-Feb		To Las Cruces	Boat out of El Campanario	Travel to Las Cruces	Revisions. Writing.
3-Feb		At Las Cruces	Orientation & reconnaissance	Revisions. Writing. Botany.	<b>Lec: Evol Ecology (DG, ACob)</b>
4-Feb	Sun	At Las Cruces	Revisions. Writing. Botany.	Revisions. Writing. Botany.	<b>Discussion: The nature of knowledge</b>
5-Feb		At Las Cruces	Revisions. Writing. Botany.	Writing. Botany practicum.	SIPs 1-4 all accepted and filed
6-Feb		To La Selva	Travel	Travel	<b>Lec: Plant-herbiv interactions (MA, AS)</b>
7-Feb		At La Selva	Orientation & reconnaissance	SIP-5 research development	SIP-5 proposals. Night walk
8-Feb		At La Selva	SIP-5 pilot studies	SIP-5 research	<b>Lec: Ecosystems (MA, CV)</b>
9-Feb		At La Selva	SIP-5 research	SIP-5 research	SIP-5 analyses. Nightwalk
10-Feb		At La Selva	Banana plantation	SIP-5 research	<b>Lec: Cons Bio 2 (MA, GC)</b>
11-Feb	Sun	At La Selva	SIP-5 research	SIP-5 analyses & context	SIP-5 symposium.
12-Feb		At La Selva	Writing SIP-5 v01 due.	SIP-5 v02, v03, filed.	Final deadline for Costa Rica papers
13-Feb		To San Jose	Travel to San Jose.	Swap gear at OTC. Recon in San Jose	Prepare for Caribbean
14-Feb		To Grand Cayman	Depart Hotel 04:30. Fly to Miami	Fly to Grand Cayman	Overnight in Grand Cayman
15-Feb		To Little Cayman	Fly to Little Cayman	Arrive Little Cayman Research Center	Orientation
8-Mar	Thu	To Miami	Fly to Grand Cayman	Fly to Miami. End of program.	

## LITTLE CAYMAN 2018 SCHEDULE

Date		Morning	Afternoon	Evening
15 Feb Thu	Group Arrives at 3:05 pm from Grand Cayman		Pick up students Unpack, get settled, and have dinner	Introduction to LCRC (Jon Clamp) <b>Discussion:</b> schedule (tentative) and expectations for LC segment.
16 Feb Fri	Orientation and General natural history	<b>Lecture:</b> Coral reefs in the Caymans and past projects (CC)  Snorkeling in back reef (Creature feature observation)	<b>Queen Conch Project</b> South Hole snorkeling  Safety instruction and gear assigning (Greg Locher, Lowell Forbes)	Creature feature <b>Lecture:</b> Introduction to Queen Conch (CC) <b>Film:</b> Secret sex life of fish
17 Feb Sat	Queen Conch project	<b>Lecture:</b> Katie Correia, Corals (8am) <b>SCUBA and Snorkeling</b> Shore dive/snorkel at Cumber's Cave	<b>Queen Conch Project</b> Data collection design Snorkeling in South Hole	<b>Lecture:</b> Paul Maneval, Coral Restoration (7:30 pm) Invertebrates and zooplankton (CC)
18 Feb Sun	Queen Conch project	<b>SCUBA and Snorkeling</b>	<b>Queen Conch Project</b> Algae sampling and identification	Data analysis and findings Creature Feature <b>Lecture:</b> Grouper Moon (Bryce Semmens video)
19 Feb Mon	Queen Conch project	<b>Queen Conch Project</b>	<b>Queen Conch Project</b> Visit other project sites	<b>Lecture:</b> Invertebrates and zooplankton (CC) <b>Queen Conch Project Presentation</b>
20 Feb Tues	Project 1 begins	Project 1 Brainstorm Project 1 exploration, design, and group members	Project 1 idea discussion and proposal development <b>Project 1 Proposal Presentation</b>	<b>Lecture:</b> Movement ecology (MBB) <b>Critique:</b> Invertebrates (Clayton Jacques)
21 Feb Wed	Project 1	<b>Project 1 Proposal due</b> Project 1 data collection	Project 1 data collection <b>Lecture:</b> Reef Nekton (CC) (5pm)	<b>Critique:</b> Herbivory (Paul Vickers) <b>Critique:</b> Coral-algae competition (Alex Stendahl)
22 Feb Thu	Project 1	Project 1 data collection	Project 1 data collection <b>Lecture:</b> Sensory ecology (DG)(5pm)	<b>Critique:</b> Sponges (Maanav Jalan) <b>Celia's Mercury Talk at Southern Cross</b>
23 Feb Fri	Project 1	Project 1 <b>Lecture:</b> Tanja Laaser Iguana Conservation (11am)	Project 1	<i>R&amp;R: Karaoke</i>



24 Feb Sat	<b>Dia libre (OFF)</b>	<b>Beach Cleanup</b>	<b>Cookout and snorkeling at Point of Sand</b>	<i>R&amp;R</i>
25 Feb Sun	Project 1 completion Project 2 begins	Project 1 – data analysis, write methods	<b>**Project 1 PRESENTATIONS**</b> (5 pm)	<b>Critique:</b> Zooplankton (Alexander Cotnoir) <b>Critique:</b> Fish ecology (Mary Pedicini) Project 1 writing
26 Feb Mon	Project 2	<b><i>SCUBA and Snorkeling</i></b>	<b>**Project 1 – 1<sup>st</sup> Draft due**</b> Visit new sites Nekton Lecture (CC) 5pm	<b>Critique:</b> Fish Biology (Grace Callahan) <b>Critique:</b> Fish Biology (Alec Cobban)
27 Feb Tues	Project 2	Project 2 Brainstorm and exploration Project 2 preliminary data collection	Finalize project 2 idea, design, and group members Project 2 data collection	<b><i>Night SCUBA and Snorkeling</i></b>
28 Feb Wed	Project 2	<b>Project 2 proposal DUE</b> Project 2 data collection	Project 2 data collection <b>**Project 1 final draft Due**</b>	<b>Lecture:</b> Climate Change and coral reefs (CC) <b>Critique:</b> Fish Behavior (Balt von Hoyningen)
1 Mar Thu	Project 2	Project 2 – data collection	Project 2 – data collection Lionfish talk (Drew Butkowski) 2pm	<b>Student Talk at Southern Cross</b> Discussion: Frustrations and Foibles of Science
2 Mar Fri	Project 2	<b><i>SCUBA and Snorkeling</i></b>	Project 2 – data collection LC Sea Turtles (Beth Chafin)	<b>Critique:</b> Coral Bleaching (Angela Ortlieb) <i>R&amp;R: Karaoke</i>
3 Mar Sat	Project 2	Project 2 – data collection	Project 2 –data analysis	<b><i>Night SCUBA and Optional Snorkeling</i></b>
4 Mar Sun	Finish Project 2	<b>**Project 2 PRESENTATION**</b> Project 2 - writing	<b>**Project 2 1<sup>st</sup> draft DUE**</b> <b>Lecture:</b> Marine plastics (CC) 1pm	<b>Critique:</b> Mangrove Fish (Hannah Hoffman) <b>Critique:</b> Reef Conservation (Corinne Victorisz)
5 Mar Mon	Finish Queen Conch write-up	Project 2 refine drafts	<b><i>Ice Cream at Debbie's (1 pm) and visit to LC Museum and National Trust (2:30)</i></b> Project 2 refine drafts	<b>Film:</b> Maug's Caldera Revisions & copy editing of all LC projects <b>Discussion:</b> Graduate School/Career
6 Mar Tues	Finish all project editing	<b>**Project 2 final draft DUE**</b> Self-Evaluations	Revisions and copy editing of all LC projects Self Evaluations	Individual meetings <b><i>Iguana hunting with Tanya</i></b>
7 Mar Wed	Clean up and pack	<b><i>Salt Rock Snorkeling</i></b> Clean up science equipment/field sites Individual meetings	Cleanup & pack Individual meetings Faculty/TA evaluations	Dinner at Beach Resort
8 Mar Thu	Depart LC 9:15 am			

## PAPERS FOR STUDENT PRESENTATIONS: COSTA RICA

#	Site	Lecture	Student	Student Paper	Staff
1	PV	Intro CR Ecol	Mary Pedicini	Janzen, D. H., and P.S. Martin. 1981. Neotropical anachronisms: the fruits the gomphotheres ate. <i>Science</i> 215:19-27.	ML
2	PV	Primate Ecol	Angela Ortlieb	Perry, S., H.C. Barrett, J. H. Manson. 2004. White-faced capuchin monkeys show triadic awareness in their choice of allies. <i>Animal Behaviour</i> 67: 165-170. And Boinske, S. 1988. Use of a club by wild white-faced capuchin to attack a venomous snake ( <i>Bothrops asper</i> ). <i>Am. J. Primatology</i> 14: 177-179.	ML
3	PV	Social insects	Balt von Hoyningen	Reid, C. R., M. J. Lutz, S. Powell, A. B. Kao, I. D. Couzine, and S. Garnier. 2015. Army ants dynamically adjust living bridges in response to a cost-benefit trade-off. <i>PNAS</i> 112:15113-15118.	ML
4	Junquilla	Sea turtles	Clayton Jacques	Shillinger, G. L. et al. 2008. Persistent leatherback turtle migrations present opportunities for conservation. <i>PLoS Biology</i> 6:1408-1416. (*See also: Crowder, L. et al. 1994. Predicting the impact of turtle excluder devices on loggerhead sea-turtle populations. <i>Ecol. Appl.</i> 4:437-445.	ML, MBB
5	MV	Avian Ecol	Maanav Jalan	Vanderbilt et al. 2015. Variation in the performance of cross-contextual displays suggests selection on dual-male phenotypes in a lekking bird. <i>Anim Behav</i> 107:213-219. (*See also: McDonald and Potts. 1994. Cooperative display and relatedness among males in a lek-mating bird. <i>Science</i> 266:1030-1032.	DG
6	MV	Diversity & Coexistence	Alexander Cotnoir	McCain, C. 2009. Vertebrate range sizes indicate that mountains may be 'higher' in the tropics. <i>Ecology Letters</i> 12:550-560. (*See also: Janzen, D. H. 1967. Why mountain passes are higher in the tropics. <i>American Naturalist</i> 101:230-243.)	MBB
7	Cuer	Coevol 1	Paul Vickers	Ramirez, S. R. et al. 2011. Asynchronous diversification in a specialized plant-pollinator mutualism. <i>Science</i> 333:1742-1746.	MA
8	Camp.	Wild card	Hannah Hoffman	Symes, L. B., R. A. Page, and H. M. ter Hofstede. 2016. Effects of acoustic environment on male calling activity and timing in Neotropical forest katydids. <i>Behav Ecol Sociobiol</i> 70:1485-1495.	MA
9	LasCruc	Evol Ecology	Alec Cobban	Reznick, D., M. Butler, and H. Rodd. 2001. Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. <i>American Naturalist</i> 157:126-140.	DG
10	LaSelv	Plant-Herbiv interactions	Alex Stendahl	Endara, M. et al. 2017. Coevolutionary arms race versus host defense chase in a tropical herbivore-plant system. <i>PNAS</i> 114:E7499-E7505.	MA
11	LaSelv	Ecosystems	Corinne Vietorisz	Anderegg, W. R. L., et al. 2015. Tropical nighttime warming as a dominant driver of variability in the terrestrial carbon sink. <i>PNAS</i> 112:15591-15596.	MA
12	LaSelv	Cons. Biol. 2	Grace Callahan	Nowakowski et al.. 2016. Infection risk decreases with increasing mismatch in host and pathogen environmental tolerances. <i>Ecology Letters</i> 19:1051-1061. (*See also Pounds, J., et al. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. <i>Nature</i> 439:161-167.)	MA

## PAPERS FOR STUDENT PRESENTATIONS: LITTLE CAYMAN

Student	Lecture	Paper
Grace Callahan	Fish biology	Miller, G.M., S. Watson, S., J.M. Donelson, M.I. McCormick, P.L. Munday. 2012. Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. <i>Nature Climate Change</i> 2: 858-861.
Alec Cobban	Fish biology	Gerlach, G., J. Atema, M. J. Kingsford, K. P. Black, and V. Miller-Sims. 2007. Smelling home can prevent dispersal of reef fish larvae. <i>Proceedings of the National Academy of Sciences</i> 104:858-863.
Alexander Cotnoir	Zooplankton	Smith, J.M., G. De'ath, C. Richter, A. Cornils, J.M. Hall-Spencer, K.E. Fabricius. 2016. Ocean acidification reduces demersal zooplankton that reside in tropical coral reefs. <i>Nature Climate Change</i> 6: 1124-1129.
Hannah Hoffman	Mangroves	Mumby, P. J., A. J. Edwards, J. E. Arias-Gonzalez, K. C. Lindeman, P. G. Blackwell, A. Gall, M. I. Gorczynska, A. R. Harborne, C. L. Pescod, H. Renken, C. C. C. Wabnitz, and G. Llewellyn. 2004. Mangroves enhance the biomass of coral reef fish communities in the Caribbean. <i>Nature</i> 427:533-536.
Clayton Jacques	Invertebrates	Carpenter, R. C. and P. J. Edmunds. 2006. Local and regional scale recovery of <i>Diadema</i> promotes recruitment of scleractinian corals. <i>Ecology Letters</i> 9:268-277.
Maanav Jalan	Sponges	De Goeij, J.M., D. vab Oevelen, M.J.A. Verimeij, R. Osinga, J.J. Middelburg, A.F.P.M. de Goeij, W. Admiraal. 2013. Surviving in a marine desert: the sponge loop retains resources within coral reefs. <i>Science</i> 342: 108-110.
Angela Ortlieb	Coral bleaching	Grottoli, A.G., L.J. Rogriguez and J.E. Palardy. 2006. Heterotrophic plasticity and resilience in bleached corals. <i>Nature</i> 440: 1186-1189.
Mary Pedicini	Fish ecology	McMahon, K.W., M.L. Berumen, and S.R. Thorrold. 2012. Linking habitat mosaics and connectivity in a coral reef seascape. <i>Proceedings of the National Academy of Sciences</i> 109: 15372-15376.
Paul Vickers	Herbivory	Dixon, D. L. and M. E. Hay. 2012. Corals Chemically Cue Mutualistic Fishes to Remove Competing Seaweeds. <i>Science</i> 338:804-807.
Corinne Victorisz	Reef Conservation	Cinner, J.E., C. Huchery, M.A. MacNeil, N.A.J. Graham, T.R. McClanahan et al. 2016. Bright spots among the world's coral reefs. <i>Nature</i> 535: 416-419.
Alex Stendahl	Coral-algae competition	Rasher, D.B. and M.E. Hay. 2010. Chemically rich seaweeds poison corals when not controlled by herbivores. <i>Proceedings of the National Academy of Sciences</i> 107: 9683-9688
Balt von Hoyningen	Fish behavior	Welch, M.J., S. Watson, J.Q. Welsh, M.I. McCormick, P.L. Munday. 2014. Effects of elevated CO <sub>2</sub> on fish behavior undiminished by transgenerational acclimation. <i>Nature Climate Change</i> 4: 1086-1089.

## MAPS: COSTA RICA AND LITTLE CAYMAN ISLAND



<http://www.nationsonline.org/oneworld/map/costa-rica-map.htm>



<http://www.southerncrossclub.com/cayman-islands/>



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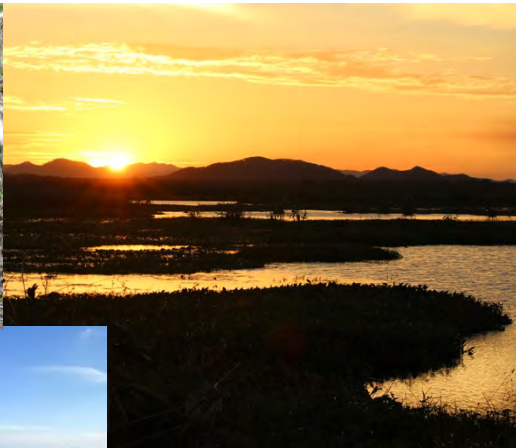


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## IT'S IN THE DIRT: ANTLION MICROHABITAT SELECTION, CONSTRUCTION, AND PREDATION SUCCESS

ALEXANDRA M. STENDAHL, PAUL J. VICKERS, AND ANGELA E. ORTLIEB

Teaching Assistants: Debora Goedert and Michael Butler Brown

Faculty Editor: Mark Laidre

*Abstract:* Organisms living in heterogeneous environments search for specific biotic and abiotic factors during microhabitat selection. To evaluate the role of soil grain size on microhabitat selection and pit construction by antlion larvae (Myrmeleontidae) we conducted the following study in Palo Verde National Park in Guanacaste, Costa Rica. Through a resource-selection survey in the wild and a multiple-choice sediment grain size selection experiment, we found that antlion larvae preferentially select soil grains < 250 microns when given the choice between soil < 250 microns, soil > 250 microns but < 500 microns, and soil > 500 microns. We also found a correlation between soil grain size and prey capture success by placing ants into antlion pits built in the three different soil types and quantifying success as a function of number of escapes before capture. When we presented antlions with unfavorable habitat options, we observed them moving large pieces of sediment away from their pits, suggesting niche construction. The findings of this study suggest that antlion larvae select for or create microhabitats with fine grain soils to maximize predation success.

*Key words:* antlion, habitat manipulation, microhabitat selection, sit-and-wait predator

### INTRODUCTION

In a heterogeneous environment, an organism's habitat selection directly impacts its fitness. Heterogeneity within an environment stems from biotic and abiotic factors that cause some habitats to be more suitable than others. There are two approaches an organism can take to optimize its habitat: choose the best environment available or construct one. Understanding how organisms weigh these factors to ultimately maximize fitness is a critical aspect of ecology.

In the context of ideal free distribution, previous studies have investigated habitat selection focusing on the impact of inter-species interactions (Rosenzweig 1991). Abiotic factors such as sun exposure, moisture, temperature, and soil type also play a significant role in the ideal free distribution of species and are crucial in identifying the viability of any environment. Organisms with limited mobility provide an excellent model system to study the impact of abiotic factors. We chose antlion larvae as our study species because they do not disperse far from where they are hatched as eggs, making microhabitat selection critical to their fitness (Linton et al. 1991).

Antlion larvae are predacious insects characterized by a sit-and-wait predatory

strategy. They build small pits in sandy soils and wait for arthropods, most commonly ants, to fall in. Once an ant is trapped in their pit, an antlion larva can throw sand from a hidden spot in the bottom of its pit at the ant to prevent escape. Previous studies have described that temperature, moisture and presence of soil crusts restrict where antlions build their pits, as these conditions impact whether a functional pit can be built (Gotelli 1993). We used Gotelli's findings to inform our questions and hypotheses regarding habitable conditions for antlion larvae.

This study investigates microhabitat selection, particularly soil grain size, as it relates the construction and performance of antlion pits. Our primary research question asked if antlion larvae prefer building their pits in a specific soil grain size. There are multiple hypotheses as to why a certain grain size might be optimal, including functionality of the pit or energy costs associated with building and maintaining a pit in a certain grain size. Functionality directly relates to how successful the pit design is at capturing a prey object. We hypothesized that certain soil types will make more effective prey traps; therefore, antlion larvae will build pits in these soil types when possible to optimize fitness, as measured by foraging success. Based on

preliminary observation of a high density of pits in fine sand in the wild, we predicted that antlion larvae may preferentially select soil types with finer grains, and that pits built in such soil types may be more successful at catching prey.

## METHODS

To evaluate soil size preference in wild populations of antlion larvae, we conducted this study at the Organization for Tropical Research in Palo Verde National Park in Guanacaste, Costa Rica in January 2018. We selected five sites around the station in which antlion pits were present. All sites were within a quarter-mile radius of the research station.

### *Observational study*

At each site, we sampled antlion pits that were at least 25 cm away from another pit and were reasonably sized and completed. We selected pits in which funnel depth was sufficient to catch an ant and the antlion was not actively building its pit. For each sample, we collected between 30 and 60 mL of soil directly from the pit and separated the samples by sediment grain size using two metal mesh sifters. Soil with grains larger than 500 microns was deemed “coarse”, smaller than 500 microns but larger than 250 microns was deemed “medium”, and smaller than 250 microns was deemed “fine”. After sifting each sample, we measured the volume of each soil type in a graduated cylinder and used these measurements to calculate proportions of each soil type present.

We then randomly selected three sites surrounding the original pit within a 25 cm radius to approximate the average dispersal of antlion larvae, as suggested by Linton et al. (1991). At each of these three sites, we collected the same amount of soil as at the pit, then sifted and measured using the same procedure. We sampled a total of 20 pits and used a paired t-test in JMP to compare the proportions of fine, medium, and coarse soil within the pit to the average proportions in its surroundings.

### *Selection experiment*

To test if antlions prefer a particular grain size, we conducted a manipulation in which we offered each antlion three soil types. For this

manipulation, we excluded sediments with a diameter greater than 4000 microns to remove excessively large pieces of gravel; other classifications for coarse medium and fine soil remained the same. We added 250 ml of each soil type to three distinct sections of a circular arena 20 cm in diameter. We then placed each antlion into the center of its own arena (n=14; Figures 1 and 2). After 15 hours, we recorded the soil type in which each antlion built its pit. We used a chi-square test of independence in JMP to evaluate significant differences from an even distribution of choices.

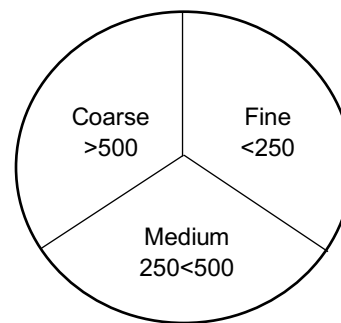


Figure 1. Diagram of selection experiment arenas



Figure 2. Multiple-choice sediment grain size arena setup for selection experiment

### *Prey capture success experiment*

To investigate the effectiveness of pits in catching prey in different soil types, we placed each antlion in a cup with 250ml of a single soil type (fine, medium, or coarse). Some of the antlion larvae we placed in coarse and medium soil had not constructed pits within the 15 allotted building hours, and, due to time constraints, we had to exclude these individuals from our experiment. We placed 3 ants into each

cup with a completed pit. We then counted the number of ant escapes (defined as an ant entering and leaving the pit) until the antlion successfully caught and killed one ant. We prepared 10 trials for each treatment, we only included analysis of cups which had pits after 15 hours. We analyzed data using a one-way ANOVA and a contrast test in JMP.

## RESULTS

### *Observational Study*

Our observational study revealed that antlion pits contained a higher proportion of fine grain soil (Fig. 3; paired-t = 8.97,  $P < 0.01$ ,  $D = 19$ ,  $SE = 0.02$ ) and medium grain soil (Fig. 3; paired-t = 5.17,  $P < 0.01$ ,  $DF = 19$ ,  $SE = 0.01$ ) and a lower proportion of coarse grain soil (Fig. 3; paired-t = -9.41,  $P < 0.01$ ,  $DF = 19$ ,  $SE = 0.02$ ) compared to the surrounding area ( $n = 20$ ).

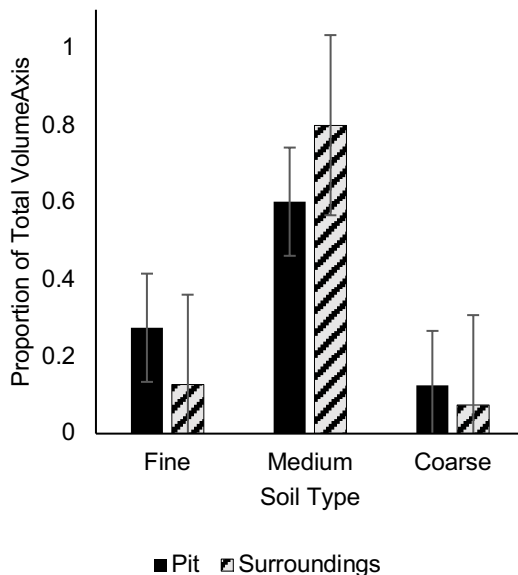


Figure 3. Grain size composition of the soil comparing antlion pits and the surrounding soil within 25 cm. Pits had a higher proportion of fine grain and medium grain soil and lower proportion of coarse grain soil. Error bars represent one standard error from the mean.

### *Selection experiment*

When given the choice of soil grain to construct a pit, antlion larvae preferentially selected the fine grain soil (Fig. 4; chi-square = 11.88,  $P < 0.01$ ,  $DF = 2$ ).

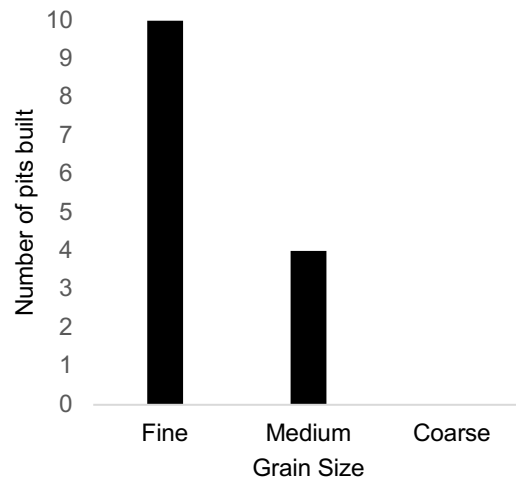


Figure 4. Antlion pit location choice when presented three distinct grain sizes. Antlion choice showed an overwhelming preference for fine grain soil and a clear avoidance of coarse soil.

### *Prey capture success experiment*

We observed a significant difference in prey capture success across soil types (Fig. 5;  $F_{2,23} = 4.37$ ,  $P = 0.03$ ). Based on number of escapes, pits in coarse soil were significantly less effective at catching prey than pits in fine (Fig. 5; Contrast<sub>1,21</sub> = 8.45,  $P < 0.01$ ) and medium soil (Fig. 5; Contrast<sub>1,21</sub> = 5.36,  $P = 0.03$ ). Pits in fine and medium soil were equally effective at catching prey (Fig. 5; Contrast<sub>1,21</sub> = 0.43,  $P = 0.52$ ).

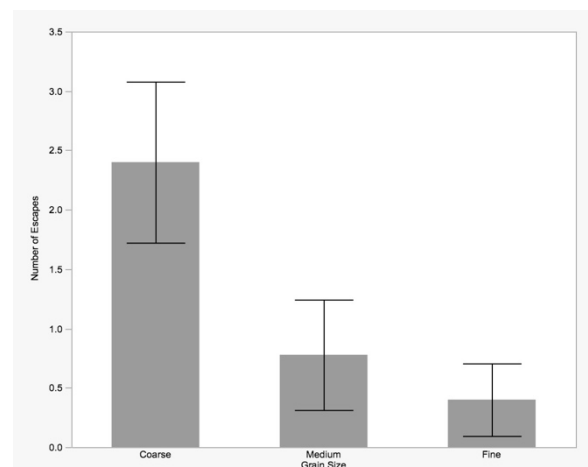


Figure 5. Average number of ant escapes from pit versus soil grain size. Pits were more effective at catching prey in medium and fine grain soils. Error bars represent one standard error from mean.

## DISCUSSION

Our results support our hypothesis that soil size affects antlion pit construction and effectiveness of pits in catching prey. In our observational study, pits contained a higher proportion of fine soil and significantly less coarse soil than their surroundings. During experimentation, antlions chose fine soil which ultimately yielded the most successful pits.

The results suggest that antlion larvae preferentially select pit location for fine sediment grain size within the limited scope of their microhabitats and that the optimal soil combination for antlion pits contains a substantial amount of fine-grained soil and a scarcity of large grained soil. We suggest two possible explanations for this result, the first being that antlions simply find the finest soil in their microhabitat (Figure 3). The other explanation stems from an unexpected observation we made while conducting our prey success experiment: we watched as several antlion larvae in the coarse soil treatment “landscaped” their cups by moving large pieces of gravel away from the site of their future pit, creating a suitable habitat where none existed before. These anecdotal observations point towards niche construction by antlion larvae, which is an exciting result that should inspire further study. Additional evidence to support this hypothesis comes from our direct observations of pits present in a gravel parking lot, in which it appeared that the antlion larvae had built their pits in the only tiny patches of fine, gravel-free soil present. This supports the idea that individuals may manipulate their environment when a suitable habitat is unavailable, i.e. in a gravel parking lot, but further study is needed to test this observed capacity.

In our selection experiment, no antlion larvae built pits in coarse soil. Four antlions did, however, build pits in medium sand. Assuming from our results that antlions are capable of selecting a soil type that they deem habitable and worthy of investing the energy to build in, it would be interesting to investigate how long antlions will search for the ‘perfect’ soil type before settling on a ‘decent’ one. Anecdotal evidence for this experiment suggests that the

majority of antlions did not move far from their starting placement, and upon encountering fine or medium soil they did not appear to continue searching before building their pit. We observed that antlions scoot around in a circle around the location of their future pit prior to building, and we suspect that this behavior could be a method of investigating the soil to verify habitability and feasibility of construction. We noted this behavior in our selection experiment as well as in our prey success experiment, in all soil types while the antlions built their pits. Further studies should look to uncover the exact processes by which antlion larvae assess pit location, construct pits, and how long they will search for suitable soil.

As antlion larvae are sit-and-wait predators who invest a significant amount of time and energy into digging their prey capture pits, it is beneficial to build pits that are the most successful in capturing prey. Our prey success experiment suggested that both the fine and medium grain sizes outperformed coarse yet performed similarly to one another in catching prey. This is an interesting result in the context of our selection experiment, in which antlion larvae consistently chose fine over medium soil. One explanation for this result is that fine soil may be easier to build in and require less energy to maintain. This hypothesis requires further testing but could yield interesting insights into secondary mechanisms of antlion microhabitat selection.

While running our prey success trials, we visually observed a trend of fewer ant escapes from antlion pits built in fine versus medium soil. When an ant falls into a pit, the antlion grabs it in its jaws repeatedly whacks the ant against the pit’s walls to kill it. We noticed that in fine soil, this violent movement caused the walls of the pit to cave in and become more vertical. Therefore, while the antlion was busy trying to kill one ant, additional ants that fell into the pit were unable to escape. This observation suggests that fine sediment grains may help antlion larvae retain prey even when an ant escapes its jaws. Future study could explore this phenomenon. Similarly, understanding the structural characteristics of different soil types that make some pits more

successful than others could illuminate how microhabitat choice works to extend fitness beyond the phenotype of the antlion itself.

Along with the abiotic factors discussed in this study, microhabitat selection by sit-and-wait predators depends upon biotic factors, including density of competitors and prey availability. According to ideal free distribution theory, individuals should distribute themselves proportionally to the supply of resources in a given area. Our results indicate the importance of abiotic factors on microhabitat selection, but future studies should investigate species interactions as they apply to this system. Similarly, one area in need of additional study is the mechanisms by which adult Myrmeleontidae choose where to oviposit, which directly impacts the habitat options of their offspring.

The results of our study provide strong evidence that sediment grain size directly influences the location of antlion pits and prey capture success. Abiotic factors play a substantial role in habitat selection on every scale and understanding how various abiotic factors inform individual decision-making can pave the way for a more complex understanding of ecological systems.

#### ACKNOWLEDGEMENTS

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#### AUTHOR CONTRIBUTIONS

All author contributed equally.

#### LITERATURE CITED

- Gotelli, N. J. (1993). Ant lion zones: causes of high-density predator aggregations. *Ecology*, 74(1), 226-237.
- Klein, B. G. (1982). Pit construction by antlion larvae: influences of soil illumination and soil temperature. *Journal of the New York Entomological Society*, 26-30.
- Linton, M. C., Crowley, P. H., Williams, J. T., Dillon, P. M., Aral, H., Strohmeier, K. L., & Wood, C. (1991). Pit relocation by antlion larvae: a simple model and laboratory test. *Evolutionary Ecology*, 5(2), 93-104.
- Rosenzweig, M. L. (1991). Habitat selection and population interactions: the search for mechanism. *The American Naturalist*, 137, S5-S28.



## HUMAN-WILDLIFE INTERACTION AND FLIGHT INITIATION BEHAVIOR IN *CTENOSAURA SIMILIS*

ALEC B. COBBAN, CLAYTON E. JACQUES, BALTHASAR L. VON HOYNINGEN HUENE

Teaching Assistants: Debora Goedert, Michael Butler Brown  
Faculty Editor: Mark Laidre

**Abstract:** Potential threats to animals can come from many predators, both familiar and novel. To expend the correct amount of energy to evade a threat, animals modify escape behavior according to perceived threat urgency. We tested how flight initiation behavior in black iguanas (*Ctenosaura similis*) differed by threat approach rate and habituation to humans. Iguanas initiated escapes at significantly greater distances when approached quickly and when approached away from frequent human activity. Iguana behavioral changes due to interactions with humans suggest that increases in human presence can cause changes to animal behaviors, with ecological consequences. These consequences could be drastic, such as reduced foraging efficiency which can change food web dynamics, or changes in mating behavior, which could isolate unique populations from one another.

**Key words:** anti-predator behavior, *Ctenosaura similis*, flight initiation distance, habituation, human-wildlife interaction

### INTRODUCTION

Interspecific animal interactions take many forms (Frid & Dill 2002). The outcome of a potentially hostile animal interaction depends upon many variables, such as the method of the approach and previous experiences of contact. Anthropogenic effects have modified the ecology of many organisms, including their recognition of and response to threats. Forms of and familiarity with human contact can alter interactions between humans and wildlife.

Habituation to human presence likely influences fleeing strategies. Flight initiation distance (FID) - the distance at which an animal flees from an approaching potential threat - is a common measurement for an organism's risk assessment of a potential danger. Inland blue-tailed skinks (*Emoia impar*) flushed with longer FID in areas with a low human presence than high presence, likely due to habituation (McGowan et al. 2014).

Fleeing behavior also appears to vary with threat urgency. A literature review conducted by Frid & Dill (2002) on the approach pace of a potential threat revealed inconsistent support for this prediction. In contradiction, however, Cooper (1997) considered theoretical cost-benefit modelling for the importance of reaction times to the rate of an approaching threat and found that the broad-headed skink (*Eumeces*

laticeps) has a longer FID when approached more rapidly. Moreover, Cooper et al. (2003) found higher FID among Bonaire whiptail lizards (*Cnemidophorus murinus*) also when approached more rapidly. These findings invite further investigation into species-specific reactions to differences in approach pace and habituation to potential human threats.

The black iguana (*Ctenosaura similis*) is an abundant lizard species native to Central America. Fear of humans and escape behavior in reaction to human approach may operate as exaptations of broader antipredator strategies: humans have historically hunted these lizards across their range, and there are enduring human-related risks (e.g. vehicles, capture by researchers) even while under hunting protection within a national park (Burger & Gochfeld 1993). How iguanas have adjusted their fleeing behavior to potential human threats informs the study of broader ecological effects stemming from a non-lethal human presence.

In this study, we investigated how black iguanas react to different levels of human contact. We hypothesized that iguanas habituate in relation to their exposure and proximity to humans. Our experimental manipulations tested the iguanas' FID in areas with higher and lower human presence. We predicted that in areas with high human activity, iguanas will have a shorter

flight initiation distance from humans, because they will be more accustomed to humans as a non-lethal threat. Emulating Cooper (1997), we further hypothesized that iguanas adjust flight initiation distance to account for the human approach rate. We varied the pace of the approaching human to gauge the changes in flight initiation distance from a slow-moving versus a fast-moving threat. We predicted that a faster approach would induce a longer flight initiation distance. The ability of the iguanas to perceive and react to humans as threats may inform how contact with humans modifies reactions to predators, with potential ecological consequences for antipredator strategies and foraging efficiency.

## METHODS

During January 2018, we observed 38 *C. similis* in Palo Verde National Park, Costa Rica: 10 iguanas along a park road, out of sight of human habitation, and the remaining 28 within sight of a park ranger station. Iguanas along the road would have experienced intermittent human contact, such as pedestrians and vehicle traffic. Iguanas at the ranger station experienced more regular human contact, including residents and drivers, as well as contact with well-maintained human habitations.

When we spotted a sedentary iguana, we observed general characteristics and behavior for 3-10 minutes, at a distance of at least 10 meters. We recorded the individual's relative size class, sex, percentage of body in direct sunlight, number of conspecifics within three meters, and head-bobbing behavior (all this data is not all analyzed here, although it was compared with flight initiation distance to rule out confounding factors). We then approached the iguana at a constant pace (slow walking or fast running). After the iguana initiated a flight response, we recorded the distance between the researcher and the iguana (flight initiation distance), as well as the distance of its initial escape (flight distance). All statistical analyses were completed in JMP Pro 13.0. Distributions of FID data were bottom-skewed, so FID data were natural-log transformed prior to performing t-tests.

## RESULTS

The mean flight initiation distance was significantly greater among iguanas sampled along the road than at the ranger station (Figure 1). Among road observations, the mean FID (absolute distance) was 9.76 meters. Ranger station observations (slow approach only) yielded a mean FID (absolute distance) of 3.53 meters.

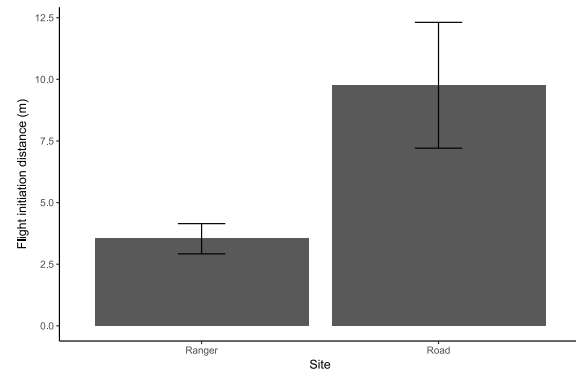


Figure 1: Flight initiation distance (meters, mean + standard error) by site (Ranger/Road). Ranger site had much higher human impact than road. Iguanas along the road displayed significantly greater flight initiation distances than did those within sight of the ranger station ( $t = 3.62$ ,  $df = 17.91$ ,  $P = 0.002$ ).

The mean flight initiation distance was also significantly greater among iguanas approached at a fast pace than a slow pace at the ranger station (Figure 2). Fast approaches produced a mean FID (absolute distance) of 6.13 meters. Slow approaches gave a mean FID (absolute distance) of 3.53 meters.

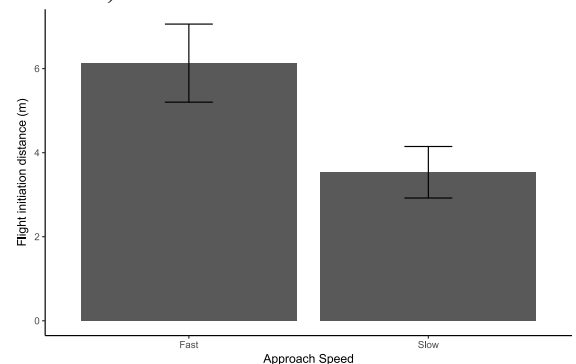


Figure 2: Flight initiation distance (meters, mean + standard error) by the speed of the approach (Fast/Slow) for iguanas sampled at the Palo Verde Ranger Station. Fast approaches had significantly greater FID than slow approaches ( $t = -2.61$ ,  $df = 21.02$ ,  $P = 0.02$ ).

## DISCUSSION

We found that iguanas in areas of high human presence had a lower flight initiation distance than those studied in areas of lower human presence. This finding is consistent with our initial hypothesis, which suggests that iguanas can habituate to non-lethal human threats.

Due to the limited scope of this project, we were unable to track individual iguanas over extended periods of time. This makes it difficult to determine if the change in flight response was truly caused by habituation or by differences in iguana activities at different sites (i.e. foraging vs. mating). Future studies could track the location of iguanas and determine how the iguana's life history is related to areas of human activity.

Short-term variation in human behavior also appears to influence iguana threat perception. In our experiment, iguanas had a higher flight initiation distance when the researcher's approach pace was faster. This is consistent with our second hypothesis: that iguanas will respond to faster-approaching threats sooner to better evade predation. This is consistent with previous research that has shown that other species of iguana respond to threats according to optimal escape theory based on perceived risk (Cooper 2003). In future studies, it may be useful to more carefully vary speed of approach and determine if iguana flight initiation distance changes proportionally to approach speed.

Iguana responses to humans can cause behavioral variability, with important possible consequences for their surrounding ecosystems. Habituation may increase foraging efficiency and/or mating success by reducing "false alarm" responses to humans. In these cases, resulting population increases could put pressure on local food sources, prompt range expansion into adjacent regions, or accelerate pathogen spread.

In these ways, human-iguana interactions could have unexpected ecological effects. A better understanding of these effects may be relevant to broader studies of human impacts on ecosystems. As human populations increase

globally, and as wildlife experience increased contact with human activity, the true consequences of that contact will become even more important.

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## AUTHOR CONTRIBUTIONS

All authors contributed equally.

## LITERATURE CITED

- Burger, J., and M. Gochfeld. 1993. The importance of the human face in risk perception by black iguanas, *Ctenosaura similis*. *Journal of Herpetology* 27(4):426-430.
- Cooper, Jr., W. E. 1997. Factors affecting risk and cost of escape by the broad-headed skink (*Eumeces laticeps*): predator speed, directness of approach, and female presence. *Herpetologica* 53(4):464-474.
- Cooper, Jr., W.E. 2003. Risk factors affecting escape behavior by the desert iguana, *Dipsosaurus dorsalis*: speed and directness of predator approach, degree of cover, direction of turning by a predator, and temperature. *Canadian Journal of Zoology* 81(6): 979-984.
- Cooper, Jr., W. E., Perez-Mellado, V., Baird, T., Baird, T. A., Caldwell, J. P., and L. J. Vitt. 2003. Effects of risk, cost, and their interaction on optimal escape by nonrefuging Bonaire whiptail lizards, *Cnemidophorus murinus*. *Behavioral Ecology* 14(2):288-293.
- Frid, A., and L. Dill. 2002. Human-caused disturbance stimuli as a form of predation risk. *Conservation Ecology* 6(1): 11. [online] URL: [www.consecol.org/vol6/iss1/art11](http://www.consecol.org/vol6/iss1/art11)
- McGowan, M. M., Patel, P. D., Stroh, J. D., and D. T. Blumstein. 2014. The effect of human presence and human activity on risk assessment and flight initiation distance in skinks. *Ethology* 120:1081-1089.

# TERMITE TUNNEL REPAIR TIMING: SPATIAL AND ENVIRONMENTAL FACTORS IN COLLECTIVE DECISION-MAKING

GRACE S. CALLAHAN AND CORINNE R. VIETORISZ

Teaching Assistants: Debora Goedert and Michael B. Brown  
Faculty Editor: Mark Laidre

*Abstract:* Efficient and cooperative response to danger is necessary for survival in a eusocial insect colony. When faced with danger, colonies decide how to respond most effectively using collective intelligence. When tunnels leading from the mound of the tree-dwelling termite species *Nasutitermes corniger* are broken, termites are deployed to repair these breaks. Here we examine termite tunnel repair times in response to factors related to colony structure and environmental influences to gain a better understanding of how termite colonies prioritize and allocate resources, and whether there are patterns that hold true across all colonies. Our results show that the mound size and the distance of the break from the mound have no influence on repair time. When alternate tunnel routes are available to circumvent the break, repair times tend to be longer. Repair times tend to be faster at night, and in some cases where alternate routes are present termites delay repair during the day and instead fix them at night. This delay indicates that colonies have a spatial understanding of their tunnel networks and can use collective intelligence to evaluate multiple variables in making repair decisions. Through this collective intelligence, termite colonies have a strong ability to prioritize and allocate resources in a way that minimizes cost to the colony.

*Key Words:* collective intelligence, termites, tunnel repair

## INTRODUCTION

In eusocial insects, high-level cooperation and group decision-making is imperative to colony survival. When a colony is threatened, its members must work together to protect most efficiently. Understanding how eusocial insects make decisions in response to danger can reveal how collective intelligence manifests.

Neotropical termites serve as a model system of eusociality in insects. *Nasutitermes corniger*, a common neotropical termite, constructs nests located in trees that contain a thick, dense interior and a less dense exterior (Thorne 1980). Extending from these nests are tunnel networks running along the tree to the ground, allowing termites to travel with protection from predators and environmental elements like humidity and sunlight. When these tunnels are broken, termites repair them to protect the nest and maintain travel.

The main selective pressure on termite nests is defense against predators (Noirot 2000). After areas close to the central mound are damaged by predators such as anteaters, termites will reinforce these areas (Lubin and Montgomery 1981; Thorne 1980).

Mound size, possibly correlated to colony size, may affect termite response to danger because larger colonies are more energetically efficient (Muradian et al. 1999). Additionally, the need to maintain homeostasis in temperature and humidity influences nest architecture (Noirot 2000). In addition to solely influencing original construction, these environmental factors such as temperature and humidity may also influence repair associated with the nest and its tunnels.

We evaluated termite response to threats to the colony in terms of structural prioritization and resource allocation by examining what factors affect tunnel repair times after damage. To assess how mound size, distance of break from the mound, presence of alternate routes, and day versus night affect repair time, we damaged the termite nest network by making breaks in the tunnels and measured their repair times.

We hypothesized that the four factors we measured will affect repair times. A larger mound size may correlate to a larger colony size, therefore resulting in a faster repair time due to higher energetic efficiency and more termites available to work on repair. We also predict that

breaks closer to the mound will be repaired faster due to the increased importance of areas closer to the nest.

The presence of alternate routes around a break will likely result in slower repair times because they will be considered less urgent and allow for continued travel. Lastly, differences in repair times between night and day could be shaped by two conditions: influence on the termite individuals and on how the temperature affects the mound. Because homeostasis in temperature and humidity are important to termite nests, repair times could be faster at night because it is a more bearable temperature for the individual termites repairing the break and they will be able to work more efficiently; alternatively, breaks may be repaired more quickly during the day because homeostasis of the mound is threatened at this time.

## METHODS

### *Experimental Methods*

We selected twelve active termite mounds located in trees along the road between the Palo Verde Organization for Tropical Studies research station and the ranger station. During preliminary observation, we measured the height and width of each mound and examined the connecting tunnel system, identifying the main tunnel leading to the ground. When multiple tunnels existed the largest and most direct was determined to be the main tunnel. We made 1 cm long breaks along these tunnels at 0.25m, 1m, and 2m from the mound. To evaluate potential influences of tunnel width on repair time, we recorded width at the break location prior to making the cuts. We recorded the time taken to repair each break, defining “repair” as the point at which we could no longer see termites. After making cuts, we regularly monitored the status of tunnels at 20 minute intervals. As repairs neared completion, we reduced these intervals to increase timing accuracy. We recorded the presence of alternate routes--tunnels which connect to the main tunnel above the break and reconnect below, allowing for avoidance of the break. Two days after making our initial cuts, we selected five mounds from our previously selected group and repeated this process at night to evaluate whether

differences between day and night affect repair time.

At some breaks made during the day, the repair time was over eight hours and we could not monitor the exact time of repair, so at these breaks we recorded repair time as 500 minutes, a conservative estimate given that repair of these breaks may have taken even longer.

### *Statistical Methods*

A regression of repair time versus mound size indicated no significant difference at each distance. Repair time was log transformed to normalize the data.

When testing whether repair time was longer when an alternate route was present, we ran a mixed model with repair time as the response variable. Repair time was log transformed to normalize the data. We put presence of an alternate route as the fixed effect variable and mound number as a random effect to account for any variance in the data caused by mound identity.

A paired t-test was run for day versus night at the same break location on each mound.

## RESULTS

Mound size and distance of the break from the mound do not affect repair time (Figure 1). A regression was run on the data at each distance: 0.25m (slope±SE: 0.022±0.26,  $P = 0.934$ ,  $R^2 = 0.0007$ ), 1m (0.084±0.20,  $P = 0.679$ ,  $R^2 = 0.018$ ), and 2m (-0.340±0.193,  $P = 0.112$ ,  $R^2 = 0.257$ ).

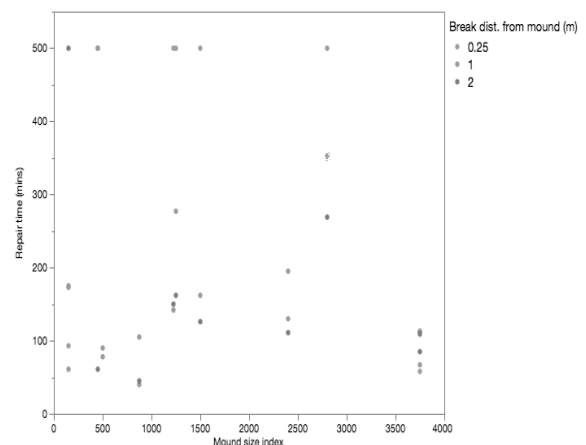


Figure 1. Repair time (mins) vs. mound size. No trends exist between mound size and repair time for different distances of breaks from the mound.

Repair time tended to be longer when an alternate route was present to circumvent the break (Figure 2) ( $P = 0.0774$ ,  $\text{Mean}_{\text{Yes}} \pm \text{SE} = 5.095 \pm 0.167$ ,  $\text{Mean}_{\text{No}} \pm \text{SE} = 4.875 \pm 0.120$ ).

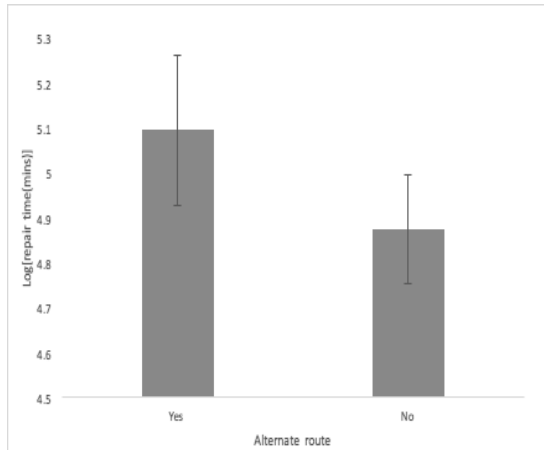


Figure 2. Break repair time  $\pm$  standard error with versus without an alternate route. Tunnel repair time tended to be longer when there was an alternate route present through which termites could circumvent the break, constant with lower repair prioritization.

Day repair times tended to be longer than night repair times (Figure 3) (paired-t = 2.023,  $df = 12$ ,  $P = 0.066$ ).

Interestingly, at three breaks made during the day in two nests the termites sealed off the breaks and then returned overnight to re-open and repair the breaks.

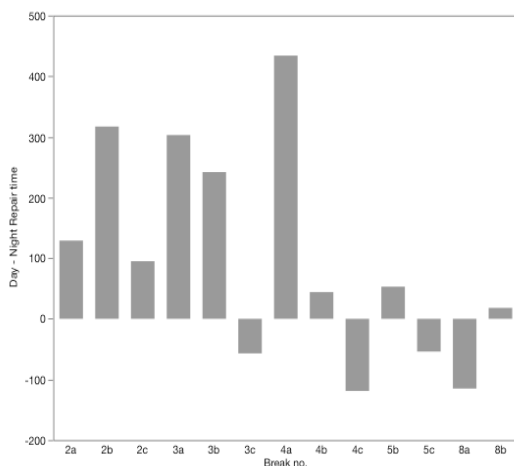


Figure 3. Difference between repair time during day vs. night. Repair times tended to be longer than night repair times. Each bar represents the day repair time

minus night repair time at a single break. On the x-axis, Break no. indicates the number of the mound the break belongs to and the letter corresponds to the distance of the break (a=0.25m, b=1m, c=2m).

## DISCUSSION

Our results show that mound size and break distance from the mound have no influence on repair time. If large and small mounds have equal ability to repair damage, which is necessary for mound survival, large and small mounds should have equal survivorship. In fact, Thorne and Haverty (2000) show that there is no difference in survivorship between large and small mounds. The lack of correlation between distance of break and repair time suggests that there is not a prioritization pattern associated with distance (i.e. breaks closer to the ground are always repaired first). However, it was rare that all three breaks in a mound tunnel system were repaired simultaneously, showing that different colonies may decide to prioritize different break distances.

Breaks with alternate routes tended to be repaired slower. This trend indicates possible spatial awareness in termite colonies, because knowledge of alternate routes can allow continued travel without exposure to the elements while expending less energy on repair. By reducing energy loss, the repair effort is a lower cost to the colony. However, each individual termite may not spatially understand the extent of the network, but their collective intelligence may be built from individual termites responding to environmental and social cues. This individual response to social cues has been seen in army ants, another eusocial insect. Army ants adjust to their environment by using information from social interaction to decide whether or not to join a bridge of ants from their group (Reid et al. 2015). Like the ants, termites may be using social cues to decide whether or not to join other termites in fixing a break.

Furthermore, breaks tended to be repaired more quickly at night than during the day. We speculate that this possible decrease in repair time at night is due to cooler temperatures and less sun exposure at night. Termites may be using environmental cues like temperature in addition to social cues to determine the best time to allocate termites for immediate break repair.

To further understand the relationship between repair time and temperature/time of day, more extensive studies need to be done. Exact temperatures should be recorded, and repair data should be gathered not only for night and day, but at different times during these periods. Humidity may also influence repair times, so trials should be run at different levels of humidity and in the presence and absence of rain.

We observed a very interesting phenomenon that indicates colony ability to balance both spatial and temporal knowledge by waiting to repair breaks until environmental conditions improved. In three instances across two different mounds, termites neither repaired nor ignored tunnels broken during the day but sealed off each end of the break. Sealing the tunnels made it impossible to travel through the tunnels, but kept the colony protected from environmental elements like temperature, humidity, and sunlight. However, the breaks were re-opened and repaired to their original status twelve hours later, after a night had passed. In each of these cases, an alternate route was present that circumvented the break. This two-fold repair suggests that in addition to spatial awareness termites may also have some type of temporal awareness since they can delay repair at non-urgent sites for a later time when conditions are better. This indicates that they are able to collectively make decisions to re-open breaks when the timing is right, even if that break occurred in the past.

Our results show that termites weigh a complex set of variables that interact to influence repair decisions. These variables can be confounded, so to understand which variables are the biggest determinants of repair behaviors and how these variables interact, tests evaluating a single variable at a time need to be done (e.g., making one break per mound at morning/afternoon/evening/night or making breaks at sites with obvious alternate routes in multiple mounds).

Our findings indicate that termites repair damage to their colonies by balancing environmental cues such as temperature and

sunlight to allocate resources most efficiently. These environmental cues may be combined with social cues, such as seeing other individuals traveling down an alternate route. Collective intelligence is the result of many individual decisions which combine to give the colony a broader spatial understanding of their tunnel systems. Using collective intelligence to effectively respond to damage with minimal costs could be true across not only termite colonies, but other eusocial insects, and reflects the necessity of a group's broader understanding of the surrounding environment for survival. Responding efficiently and partitioning resources in a cost-effective manner is critical for the survival of any group, whether it is termites, eusocial insects, vertebrates, or even humans.

#### ACKNOWLEDGEMENTS

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#### LITERATURE CITED

- Lubin YD, Montgomery GG. 1981. Defenses of *Nasutitermes* termites (Isoptera, Termitidae) against tamandua anteaters (Edentata, Myrmecophagidae). *Biotropica*. 13(1):66-76.
- Muradian R, Issa S, Jaffe K. 1999. Energy consumption of termite colonies of *Nasutitermes ephratae* (Isoptera: Termitidae). *Physiology and Behavior*. 66:731-735.
- Noirot C. 2000. Termite Nests: architecture, regulation and defence. Kluwer Academic Publishers. 121-139.
- Thorne BL. 1980. Differences in nest architecture between the neotropical arboreal termites *Nasutitermes corniger* and *Nasutitermes ephratae* (Isoptera: Termitidae). *Psyche*. 87(3-4):235-243.
- Thorne BL, Haverty MI. 2000. Nest growth and survivorship in three species of neotropical *Nasutitermes* (Isoptera: Termitidae). *Environmental Entomology*. 29(2):256-264.



## TERMITE TERMINALS: PATHWAY ORGANIZATION BY COLLECTIVE INTELLIGENCE

MARY M. PEDICINI

Teaching Assistants: Debora Goedert and Michael B. Brown

Faculty Editor: Mark Laidre

*Abstract:* Arboreal termites (*Nasutitermes corniger*) construct covered paths to prevent desiccation while foraging. The efficiency of their choices in building and repairing these paths is essential to their continued survival and requires effectual use of collective intelligence. When confronted with obstacles, termites in Palo Verde National Park in Costa Rica are here shown to distinguish between and follow the shortest and thus most efficient path around obstacles. I constructed a suite of obstacles from tape, placed them in breaks in pre-existing termites' paths, and evaluated the efficiency of their choices in rebuilding. The experimental results clearly demonstrated that termites almost always build the most efficient path around an obstacle. That termites are so adept at building along the most efficient routes is an endorsement which should encourage humans to consider the strategies of collective intelligence in our own problem-solving efforts.

*Key Words:* collective intelligence, *Nasutitermes*, optimization, path-building, self-organization, termites

### INTRODUCTION

Social insects, like stock markets, make up complex systems whose success depends completely on a series of individual-level behaviors. Their unique and fascinating system of problem solving through collective intelligence has myriad applications in human technology, from artificial intelligence to understanding of animal navigation (Reid et al. 2015) to use in computer science regarding the dynamics of self-organization (Andara et al. 2004). Social insects survive adversity through feats of self-organization, from the floating rafts made by fire ants *Solenopsis invicta* to the living chains formed by *Oecophylla* weaver ants (Reid et al. 2015). Unlike these nomadic ant species, the arboreal termites of the genus *Nasutitermes* build and fiercely defend stable home structures (Levings and Adams, 1984). Because of this, their collective intelligence is tested most in the efficiency with which they build and repair paths. These paths are crucial to their survival, protecting them from predators and maintaining homeostasis in both temperature and humidity (Noirot and Darlington, 2000). When the path is disturbed, termites must bear the extra cost of any time and energy spent building unnecessarily, so any wanton behavior would most likely have been penalized and eradicated by natural selection.

The present study examined how well termites display collective intelligence when

path-building. I predicted that termite colonies would be able to consistently select and build the shortest path around any obstacle in their way. I presented the termites with five different arrangements of obstacles, each of which allowed for multiple potential routes, but which included one (or two equivalent) objectively shorter paths. Because there was always a possibility of a more or less efficient choice, the path the termites' eventually chose would tell me if they actually did distinguish the most efficient route available. In testing this hypothesis, every failure by a colony to choose the most efficient path will reveal insight into the limits of these super-organisms' collective intelligence.

### METHODS

To evaluate the efficiency of termite tunnel repair decisions, I constructed a suite of artificial obstacles (Figure 1). I began with obstacles placed directly in the middle of the path. The arrangements dubbed "Center" and "2x Center" allowed me to determine a) if the termites responded to the tape obstacles by altering their paths, and b) if they tended to display a side bias (preferring significantly to make paths either to the left or right). I then continued with configurations with stronger disparities between choices: "Left Asymmetric" (L Asym) (the more efficient choice being to go right), "Right Asymmetric" (R Asym) (the more efficient

choice being to go left), and “2x Alternating” (2x Alt) (the more efficient choice being to go straight between the two obstacles).

I conducted this study in Palo Verde National Park in Costa Rica. Walking the main road to the left of the station as well as every side trail coming off of it for approximately 1km, I scanned for termite nests and paths. When I discovered one, I quickly created an obstacle out of folded tape, and then created a break approximately 1.5 inches long in the termite path by scrubbing my thumb across it. In the center of this break I placed the obstacles, in the appropriate configuration.

I returned to all of the sites periodically over the course of three days, and once it was clear that the path had been finished (no open spots, no termites visible in the area), I recorded if the termites had rebuilt around the obstacle to the left or right, had rebuilt both paths, or had built a path straight through the obstacles. Both left and right were considered as the most efficient choice, since the left- and right-hand paths were equal in length and thus equally efficient. While alone either of these choices were counted as most efficient, the two nests which chose both paths, forming a circle around the obstacle, were counted as making a less efficient choice because they chose a path which was longer than necessary to rebuild the tunnel. When one nest failed to rebuild at the spot I had broken after three days, I recorded it as abandoned and ended the observation. Finally, I conducted a chi-square test for each of the three arrangements with disparate choices, categorizing the possible routes as optimal or not optimal for the arrangement in question.

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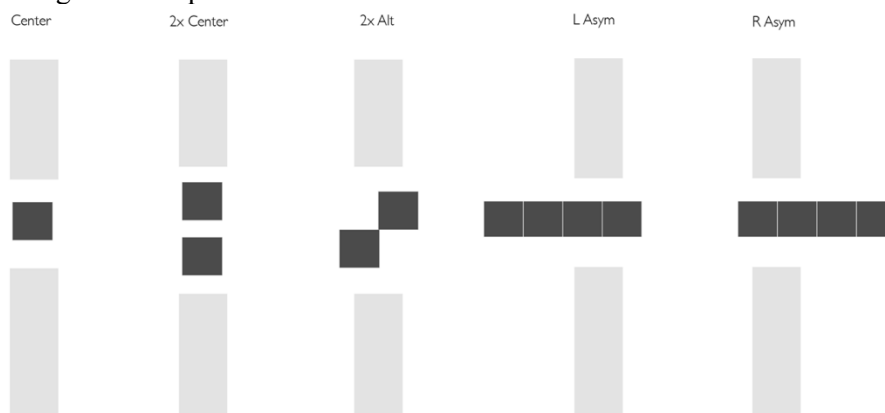


Figure 1. Arrangements of the five tests performed on termite paths, consisting of 3 dimensionally folded squares of tape (black) inserted into breaks made in pre-existing termite paths (pale grey). Center (n=8), 2x Center (n=2), 2x Alternating (n=11), Left Asymmetrical (n=8), Right Asymmetrical (n=10).

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## RESULTS

In evaluating termite responses to the centered obstacles, I found no side bias. The termites of three colonies (38%) went left, two went right (25%), two constructed both paths (25%), and one did not rebuild the path (13%). When presented with a L Asym arrangement, 100% of tested nests chose to go right, following the more efficient path (chi-square = 6.00,  $P = 0.01$ ,  $df = 1$ ). When presented with a R Asym arrangement, 90% of colonies made the more efficient choice to go left, but one colony chose both paths (chi-square = 6.40,  $P = 0.01$ ,  $df = 1$ ). They formed the left path in the same way as the other colonies had, building right up against the

tape for the shortest possible path within the left-hand choice, but constructed another path on the right-hand side as well. This path was farther out from the obstacle, beginning on the upper end exactly where I had made the initial break, but finishing on the lower end about two inches down from the initial break (Fig. 2).



Figure 2. Less efficient path choice in R Asym set-up. Colony formed both right- and left-hand paths.

Finally, ten of eleven colonies (90.91%) formed paths straight through the middle of the obstacles in the 2x alternate arrangement, making the most efficient choice (chi-square = 7.36,  $P = >0.01$ ,  $df = 1$ ). The one colony which deviated (9.09%) formed a path around both obstacles to the left (Fig. 3).



Figure 3. Less efficient path choice in 2x Alt set-up. Colony formed a path to the left of both obstacles (a longer choice) rather than through the middle of the obstacles (the shortest choice).

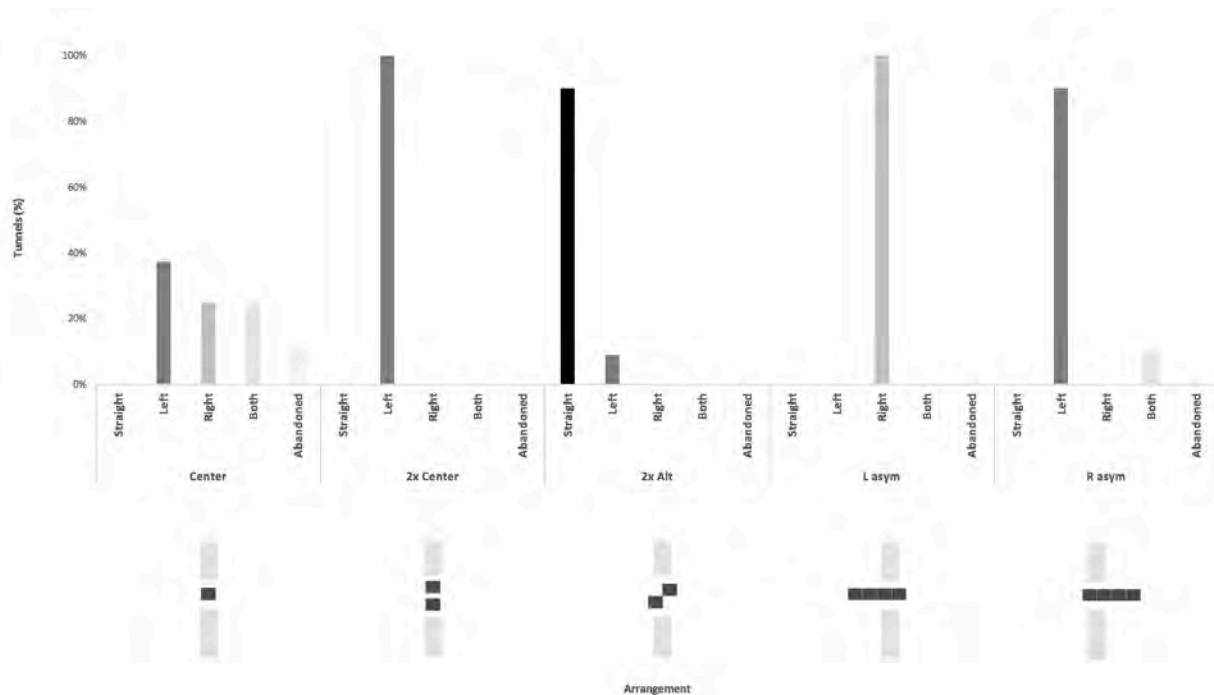


Figure 4. Percentage of colonies that chose each directional option when faced with five different arrangements of obstacles. Colonies nearly always selected the most optimal path.

## DISCUSSION

When clear disparities existed in length and therefore efficiency, the termites almost always identified the shortest path and rebuilt along it. These data support the hypothesis that termites can and do optimize efficiency in path-building. Through the choices of individuals whose own awareness is local and unconcerned with colony-level optimization, colonies were able to minimize the cost of rebuilding by choosing the shorter path to build on almost every time. This means that individuals were able to differentiate between the potentially available routes, that they identified the shortest one, and that they acted on that identification to build the most efficient path. In all of the tests conducted, there were only two times the most efficient path was not taken – once because the path was never repaired at all, and once, I suspect, because of a methodological error by which the obstacles were placed on the path in question too closely together for the termites to rebuild between them.

More interesting were the cases in which the most efficient path was taken, but it was either

taken twice, as in the case of two of the Center tests, or taken alongside another longer path, as in the case of the R asym test in which both left- and right-hand paths were taken. This behavior might reflect a failure in efficiency, but it might have a beneficial use as well. The creation of an alternate route might arise as an effort to alleviate high levels of traffic in the area. Selection may have allowed this seemingly superfluous expenditure to survive because the secondary route provides a benefit in case of a repeated disturbance.

While this study has found these termites to be clearly able to distinguish and build the most efficient path available, our understanding of the mechanisms by which these paths are decided upon warrants further investigation. In observing the termites' behavior after inserting the obstacle, I often saw termites individually exploring all available path choices. I have watched what seemed to be a use of alternate paths to regulate traffic flows in the early reconstruction around a Center obstacle. In that instance, as both workers and soldiers streamed along the left-hand path, the termites themselves

made small breaks in the path wall about two inches above and below the borders of the break I made, from which workers were seemingly funneled to the rebuilding site by soldiers moving back and forth along the line. I also noted multiple termites of varying castes seem to vibrate back and forth in place, which I suspect to be a signaling behavior. Further studies on this phenomenon might investigate the significance of this behavior in path choice, or the respective roles of various caste members.

The power of collective intelligence lies in its' ability to optimize the behavior of a complex whole based on a few simple rules operating at the level of the individual. While the mechanisms by which termites create these paths would benefit from further elucidation, I here showed a strong indication of the effectiveness of termite's collective intelligence. That these organisms are so adept at building along the most efficient routes is an endorsement which should encourage humans to consider the

strategies of collective intelligence in our own problem-solving efforts.

#### ACKNOWLEDGEMENTS

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#### LITERATURE CITED

- Andara, C., Issa, S., & Jaffé, K. (2004). Decision-making systems in recruitment to food for two Nasutitermitinae (Isoptera: Termitidae). *Sociobiology*, 44(1), 139-152.
- Reid CR, Lutz MJ, Powell S, Kao AB, Couzin ID, Garnier S. Army ants dynamically adjust living bridges in response to a cost–benefit trade-off. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;112(49):15113-15118. doi:10.1073/pnas.1512241112.
- Noirot C., Darlington J.P.E.C. (2000) Termite Nests: Architecture, Regulation and Defence. In: Abe
- T., Bignell D.E., Higashi M. (eds) Termites: Evolution, Sociality, Symbioses, Ecology. Springer, Dordrecht

## AGGREGATION DYNAMICS: SEXUAL DIVERGENCE OF TREEHOPPER GROUPING BEHAVIOR

ALEXANDER W. COTNOIR, HANNAH I HOFFMAN, AND MAANAV J. JALAN

Teaching Assistants: Michael B. Brown and Debora Goedert  
Faculty Editor: Mark Laidre

**Abstract:** Intraspecies groups commonly arise to benefit individuals involved. Although treehoppers (*Umbonia crassicornis*) are thought to aggregate based on the selfish herd hypothesis, the species' sexual dimorphism and sexually-divergent parental care behavior suggest that Bateman's paradigm operates within the system. Natural group sizes and sex ratios were analyzed to determine if an optimal group size is reached, as the selfish herd hypothesis would predict but Bateman's paradigm would not, and if an optimal male to female ratio less than one is reached, as Bateman's paradigm would predict but selfish herd hypothesis would not. We translocated an intact group to a new branch in the field and created same-sex branches to investigate the importance of grouping, parental care, and presence of the opposite sex for males and females. *Umbonia* did not exhibit an optimal group size but did exhibit an optimal male to female ratio of roughly 0.56; all translocated females returned to their original branch while only one of fourteen males did; and males dispersed more quickly from same-sex branches than females. Together, these findings indicate that Bateman's paradigm controls *Umbonia* aggregation dynamics, with males driven by female presence and females driven by resources, parental care, and a greater tendency to be in a herd.

**Key Words:** Bateman's paradigm, grouping behavior, selfish herd, *Umbonia crassicornis*

### INTRODUCTION

Intraspecies groups often arise in nature, from layers of phytoplankton to herds of wildebeest. Grouping behavior can have many adaptive functions, such as hunting, mating, and protection. However, grouping also poses several risks, such as competition and disease transmission. Therefore, group formation requires a balance of costs and benefits that influences individual behavior.

*Umbonia crassicornis*, a central American species of the family Membracidae, forms dense groups on young branches of several tree species in order to feed upon sap and to raise nymphs (Wood 1974). Individuals of all ages comprise these groups. Wood (1974) describes treehopper grouping as the byproduct of female ovipositioning behavior: female treehoppers place their eggs on a branch in a highly-concentrated pattern, generating a colony of nymphs that does not disperse before maturation (Wood, 1974). Mothers stay clustered around these nymph colonies. While this behavior explains the existence of nymphal and female groups, it does not address the prominent existence of mixed-sex adult groups.

One theory that could explain treehopper grouping is the selfish herd hypothesis, which suggests that organisms tend toward an optimal group size that balances the benefit of predator protection with the cost of resource competition (Hamilton 1971, James et al. 2008). This protection can arise from increased probability of predator detection and from having conspecifics physically block one another from predators (James et al. 2008). As observed in European minnows, predators might also struggle to target individual prey in a group, resulting in higher survivorship (James et al. 2008). According to Wood (1974), treehoppers could similarly form clusters "to deter a potential predator, or perhaps enhance cryptic resemblance; however, experimental evidence is lacking."

Another factor that could influence treehopper aggregation dynamics emerges from Bateman's paradigm, which contends that "males are limited by the availability of females, and females are limited by... provisioning for offspring" (Bateman 1948). In this paradigm, male reproductive success increases linearly with an increasing mate pool, while female reproductive success increases when more resources are present (Bateman 1948). This

discrepancy in mating success criteria can result in differences between sexes such as sexual dimorphism and divergent grouping behavior (Tang-Martinez, 2000). Wood (1974) observed that *Umbonia* males have flattened thorns and do not group based on parental care, while females have pointed thorns and do make grouping decisions based on parental care. These findings indicate that *Umbonia* may exist within Bateman's paradigm.

We tested whether the selfish herd hypothesis or Bateman's paradigm drives *Umbonia crassicornis* aggregation dynamics. The selfish herd hypothesis considers every treehopper as a unit of the aggregation without accounting for intragroup differences such as sex. Because *Umbonia* male and females appear and behave differently, they must balance not only protection and resource limitations but also mating efficacy; therefore, the species should not follow the selfish herd hypothesis. In groups driven by Bateman's paradigm, *Umbonia* should not aggregate in an optimal group size as the selfish herd hypothesis predicts. *Umbonia* should also aggregate in a stable male to female ratio equal to or less than one, as Bateman's paradigm posits that males are limited by females. Lastly, *Umbonia* should aggregate differently depending on sex, with females favoring resource-rich sites where they oviposit and males favoring presence of females over any other factor, as Bateman's paradigm would suggest.

Our results provide insight into how Bateman's paradigm might operate over the selfish herd hypothesis to give rise to the emergent properties of *Umbonia* aggregations.

## METHODS

### *Survey: group sizes and sex ratios*

We identified four systems of treehoppers (defined as all treehopper clusters in a grouping of adjacent trees) in acacia trees along the road of the biological research station in Palo Verde National Park, Costa Rica (Figure 1). To evaluate aggregation compositions, we collected group size and sex ratio summary statistics by marking a given group's branch with nail polish every three centimeters and subsequently counting the number of adult males and adult females in each segment (Figure 2). We verified

the precision of this counting tactic by polish-marking every individual on a branch we had just counted and ensuring that we tabulated the same quantities in both counts (Figure 2). Nail polish does not seem to impact aggregating behavior, as we polish-marked a male and female, and both individuals remained in their original groups and were surrounded by a similar density and composition of conspecifics the next day.

We compared the variability of sex ratios for groups smaller than 100 individuals and groups larger than 100 individuals by conducting an unpaired t-test on groups' absolute deviations from the average ratios.

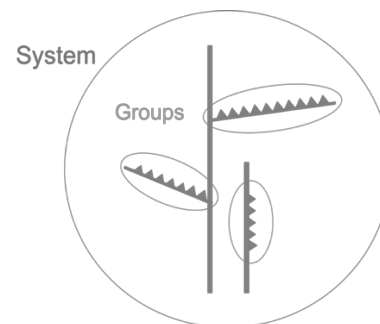


Figure 1. Categorization of "systems" and "groups" of treehoppers. A "system" refers to all treehoppers in adjacent trees, while a "group" refers to all treehoppers on a single branch.

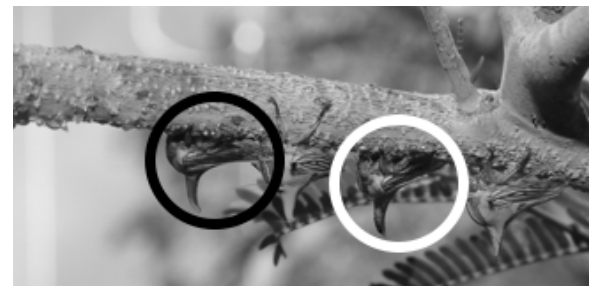


Figure 2. Photograph depicting the treehopper group size counting technique, with marked three-centimeter branch segments and polish-marked male (white) and female (black). Males are distinguishable by a flattened thorn, while females have a pointed thorn. This photograph also demonstrates our test to verify the counting technique, for which we completed a normal count and subsequently recounted while polish-marking each individual.

### *Group translocation*

To observe potential differences in male and female dispersal patterns, we randomly chose one of the 19 groups, polish-marked its 20 members



(6 females, 14 males), and arranged the group in its original pattern on a new branch three meters away, using metal forceps to pick them up by their horns. This new branch belonged to the same tree species and had similar length, diameter, and height as the original. After 24 hours, we counted the number of marked treehoppers on both the original branch and the branch to which the group had been moved.

#### *Same-sex branches: field manipulations*

To study disparity in dispersal rates of male and female treehoppers, we randomly chose one of the 18 remaining groups, polish-marked 10 of its adult males and 10 of its adult females, and created same-sex branches that had the same density as the original group in terms of individuals per three-centimeter segment. The two branches were roughly three meters from the original branch, one meter apart, belonged to the same tree species as the original, and had similar length, diameter, and height as the original. We employed the same translocation technique as used previously. We counted the number of individuals remaining in the two groups after 5, 10, 30, and 50 minutes. We conducted a complementary lab experiment to replicate this manipulation with more frequent observation points and a longer monitoring period.

#### *Same-sex branches: lab manipulations*

Aiming to study sex dispersal rates in a controlled environment, we created two enclosures by affixing two horizontal branches 10 centimeters apart halfway up 86-centimeter-tall butterfly bait traps. Branches were taken from the same tree species on which a randomly-selected system was found. The enclosures were hung 0.5 meters apart on a tree that provided similar shade as the system's original tree. We arranged ten males in the center of each branch in one enclosure such that they had the same density as their original groups in terms of individuals per three-centimeter segment. A second enclosure was created with females in the same arrangement. We recorded the number of individuals on the branches in each enclosure after 5, 10, 15, 20, 25, 30, 60, 120, 240, 960, and 1140 minutes.

## RESULTS

### *Group sizes and sex ratios*

Treehopper group size (number of adult males and adult females) follows a positively-skewed logarithmic distribution as opposed to a bell-shaped distribution (Figure 3). Groups most frequently had 10 to 20 individuals and ranged between 4 to 175 individuals (Figure 3).

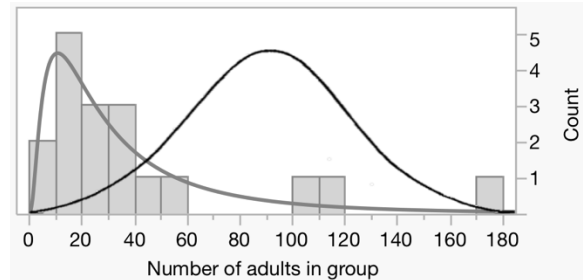


Figure 3. Distribution of treehopper (*Umbonia crassicornis*) group size in Palo Verde National Park, Costa Rica. A logarithmic trend line (grey) has been fitted to the figure, and a bell-shaped curve (black) has been superimposed to demonstrate the non-normality of the distribution. (N=19 branch groups in 4 separate systems)

Groups smaller than 100 had male to female ratios ranging from 0.12 to 1.91, while groups larger than 100 individuals had ratios ranging from 0.51 to 0.59 (Figure 4). Systems were larger than 100 individuals with male to female ratios ranging from 0.59 to 1.03 (Figure 4). Absolute deviations from the mean were significantly greater for ratios of groups smaller than 100 individuals ( $\mu=0.39$ ,  $SD=0.29$ ) than those of groups larger than 100 individuals ( $\mu=0.36$ ,  $SD=0.02$ ) ( $t(16)=2.12$ ,  $p<0.05$ ) (Figure 4).

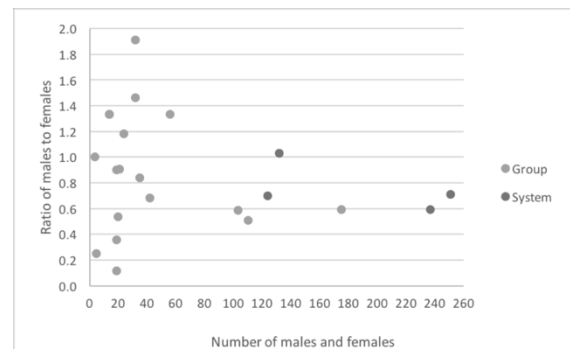


Figure 4. Male to female treehopper (*Umbonia crassicornis*) ratios as a function of group size (light grey) and system size (dark grey) in Palo Verde National Park, Costa Rica. (N=19 branch groups and 4 tree systems).

### Group translocation

Of the 6 females and 14 males translocated, all females and one male returned to the original branch after roughly 24 hours. The remaining 13 males left the system.

### Same-sex branches: field and lab experiments

All males and only 30% of females placed on same-sex branches in Palo Verde National Park, Costa Rica left the group after 50 minutes (Figure 5a). All males and females placed on same-sex branches in artificial habitats left their groups. However, while males left in under 30 minutes, females took 1140 minutes to disperse. (Figure 5b).

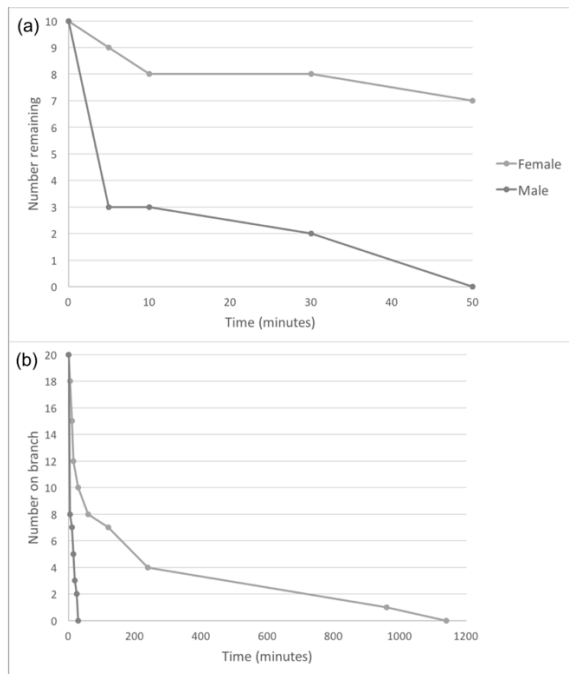


Figure 5. Number of male and female treehoppers (*Umbonia crassicornis*) remaining on branches with same-sex groups over time. Males (dark grey) and females (light grey) were placed (a) on separate branches in Palo Verde National Park, Costa Rica (field), or (b) on two branches in same-sex net enclosures (lab), and the number of each sex remaining on branches was recorded at the displayed time intervals. ((a) N=10 males and 10 females per branch; (b) N=20 males and 20 females with 10 individuals per branch)

## DISCUSSION

This study demonstrates that Bateman's paradigm drives *Umbonia crassicornis*

aggregation dynamics rather than the selfish herd hypothesis.

The selfish herd hypothesis predicts the existence of an optimal group size as individuals seek to join the largest possible group without becoming resource-limited. However, the positively-skewed group size frequency histogram demonstrates that a *Umbonia* optimal size does not exist (Figure 3).

Bateman's paradigm explains the finding that large *Umbonia* groups (>100 individuals) reach a stable average sex ratio of roughly 0.56 (Figure 4). The sex ratios for such groups are significantly less variable than ratios of groups with fewer than 100 individuals (Figure 4). This stable sex ratio skewed towards females upholds the key principle of Bateman's paradigm that males are limited by females. The observed convergence towards a consistent sex ratio potentially reflects differing stabilities of small and large *Umbonia* groups. Males might be unable to assess sex distribution if small groups disperse rapidly; on the other hand, males might be able to detect sex cues in large, stable groups in order to decide whether to join. Future studies could investigate how treehoppers consistently reach this system-level, stable sex ratio.

The translocation study also suggests that Bateman's paradigm dictates *Umbonia* group dynamics, as all females and only one of 14 males returned to their original branch. As Bateman's paradigm predicts, parental care — exhibited here in the form of site fidelity — drives females and not males, who tend to disperse to new branches in search of mates.

Same-sex branch experiments also suggest discrepancies in male and female aggregation behavior, indicating that sexual selection under Bateman's paradigm is a primary driving force in *Umbonia* aggregations. In the field, only 30% of females dispersed from an all-female branch in the time that every male had dispersed from an all-male branch (Figure 5a). Similarly, females took roughly 3800% more time to completely disperse from same-sex lab branches than males (Figure 5b). The fact that males dispersed more quickly suggests a competitive benefit for this sex to immediately flee from all-male groups in search of mates, while females more heavily value aggregating around food (i.e. branch sap)

and surrounding themselves with conspecifics. If treehoppers were driven by the selfish herd hypothesis, both males and females would have stayed in their groups or dispersed at equal rates. Along with field and experimental data, *Umbonia* sexual dimorphism provides evidence that sexual selection under Bateman's paradigm operates in the species. As the paradigm suggests, individuals whose phenotypes help them attract or compete for potential mates produce more offspring, effectively increasing those traits in subsequent generations.

We encountered challenges during data collection and experimentation that could have impacted results. Firstly, group size and sex ratio data are only snapshots of systems. Long-term monitoring of group composition could resolve this issue. Secondly, our study does not account for the nuances of *Umbonia* life stages. We did not make age distinctions that could impact results, as various male and female age classes might exhibit distinct grouping behaviors. For instance, subadult females who have not oviposited might display less site fidelity than those who have. Similarly, we did not consider that the four systems might have existed in various temporal stages of colonization (e.g. recently inhabited, recently sexually-mature, past sexual maturity), and stages could be associated with unique sex ratios, site fidelity behaviors, and propensities to disperse. Future studies could replicate the outlined experiments while accounting for maturation.

Lastly, we seeded only ten individuals on each branch. This group size might have been too small for selfish herds to form, resulting in the rapid dispersal behavior observed. Future studies could utilize larger group sizes to account for potential interactive effects between group size and sex ratio.

Little research exists regarding *Umbonia crassicornis* aggregation dynamics, and this study presents several opportunities for future study. Testing alternative theories guiding treehopper aggregations such as nutrition and thermoregulation will help develop a more complete understanding of their grouping behavior. Moreover, future studies could provide useful guidance in locations such as Florida,

where the species is invasive. For example, investigating how male *Umbonia* assess the presence of females and make grouping decisions might lead to novel methods for reducing copulation.

Despite limitations, this study presents trends in sexually-divergent grouping behaviors consistent with *Umbonia crassicornis* natural history. Ultimately, Bateman's paradigm controls aggregation dynamics in the species, a finding that challenges widely-accepted models of grouping based on protection from predation. Future studies should analyze intragroup social interactions as factors in grouping behaviors.

#### ACKNOWLEDGEMENTS

We would like to thank Mark Laidre, Michael B. Brown, and Deb Goedert for their advice and moral support, Matt Ayres and Dartmouth College for providing supplies and opportunities, and the staff at Palo Verde Biological Station for housing.

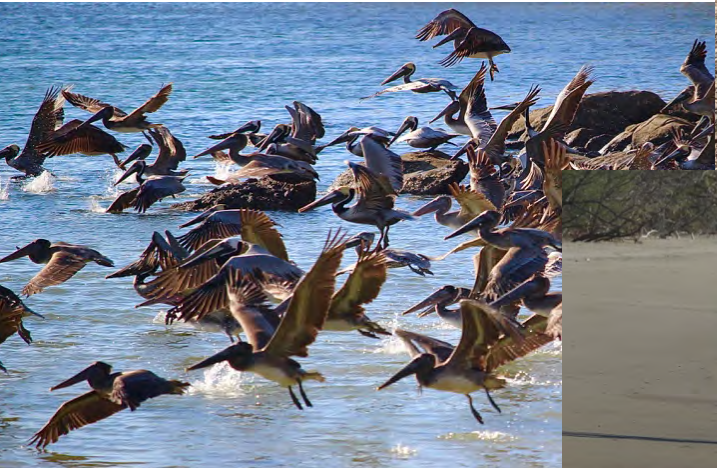
#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

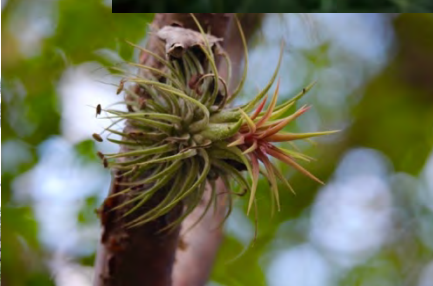
#### LITERATURE CITED

- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. *Heredity* 2: 349-368.
- Hamilton, W. D. 1971. Geometry for the Selfish Herd. *Journal of Theoretical Biology* 31: 295-311.
- Orpwood, J. E., Magurran A. E., Armstrong, J. D., and Griffiths, S. W. 2008. Minnows and the Selfish Herd: Effects of predation risk on shoaling behavior are dependent on habitat complexity. *Animal Behavior* 76(1): 143-152.
- Tang-Martinez, Z. 2000. Paradigms and primates: Bateman's principle, passive females, and perspectives from other taxa. Pages 27-32 in S. Strum and L. Fedigan (eds.), *Primate encounters: Models of science, gender, and society*. University of Chicago Press, Chicago.
- Wood, T. K., 1974. Aggregating behavior of *Umbonia crassicornis* (Homoptera: Membracidae). *Canadian Entomologist* 106: 169-173).











## COATIS AND AGOUTIS EXHIBIT NEOPHOBIC DIETARY PATTERNS

ANGELA E. ORTLIEB AND ALEXANADRA M. STENDAHL

Teaching Assistants: Michael Butler Brown and Debora Goedert

Faculty Editor: Matthew P. Ayers

**Abstract:** Neophobia, the fear of novel situations, could be an evolutionary tactic to avoid potential harm to an individual. To examine the prevalence of food neophobia among scavenging mammals that often feed in local compost piles, we conducted a randomized food preference experiment on white-nosed coatis (*Nausua narica*) and agoutis (*Dasyprocta punctata*) at the Monteverde Biological Research Station, Costa Rica. To create novel and familiar-scented foods, we marinated cubed pears in one familiar scent (mango-pineapple juice) and one novel scent (clove-peppermint). Both of our study species apparently use olfactory cues to assess food quality in this system. Coatis and agoutis both exhibited food neophobia; all coatis (n=42) and all but one agouti (n=8) initially chose to consume familiar-scented fruit when presented with both familiar-scented fruit and novel-scented fruit. Coatis showed greater reluctance to consume novel-scented fruit than agoutis. We attribute these species-dependent differences to variation in foraging techniques and social behavior. Our results provide insights into the neophobic tendencies of scavenging mammals.

**Key words:** agouti, coati, food neophobia, mammal foraging, social learning

### INTRODUCTION

When faced with a novel food item, animals must weigh the costs of consuming a potentially dangerous food with the benefits of consuming it. Food neophobia, the fear of trying novel food, results from the perceived costs outweighing the potential benefits for an individual. Often, food neophobia manifests as an initial avoidance of an unfamiliar food followed by investigation and a taste test. If there are no adverse effects within a few hours, the animal will likely return and eat with more confidence (Mitchell 1976). Similarly, an animal that encounters a high number of unfamiliar foods is more likely to consume a novel food item later (Launchbaugh 1997). Additionally, current nutritional needs and food availability may cause an individual to be neophobic or neophilic (Rozin 1968).

Food neophobia is especially important in foraging animals because determining the legitimacy of a potential food item is a recurring decision. Animals rely on various senses such as olfaction to locate and evaluate food quality (Rozin 1976). Coatis (*Nausua narica*) and agoutis (*Dasyprocta punctata*) living in Monteverde, Costa Rica are both foraging mammals with wide possible diets. Coatis are omnivores; their diet mainly consists of fruit, vegetables, and insects, but can also include eggs and meat (Alves-Costa 2004). The diet of

agoutis mainly consists of fruit and plant matter but can also include animal matter (Henry 1999). Both species use olfactory cues to locate food and exhibit dietary changes according to season and local food availability. These foraging habits make the species good subjects for studies of food neophobia.

To examine neophobia in relation to novel olfactory cues, we designed a randomized food preference experiment in which we presented coatis and agoutis with familiar and novel-scented fruit. Our primary research question evaluated the willingness of individuals to eat novel-scented fruit. We proposed two competing hypotheses to predict either a trend towards neophobia or neophilia in each species. Our primary hypothesis posited that since these animals are accustomed to an abundant food source of human compost and likely have little reason to take dietary risks, they may exhibit neophobic tendencies when presented with both a novel-scented food and a familiar-scented food. We predicted that both coatis and agoutis would first consume familiar-scented fruit, and furthermore, decline to consume any novel-scented fruit. We also considered an alternative hypothesis under the assumption that the presence of human compost regularly exposes animals in this system to novel foods, so they might exhibit higher neophilic tendencies. Under

this model, we would expect coatis and agoutis to show little aversion to novel-scented fruit. We additionally examined whether coatis and agoutis exhibit different degrees of neophobia when assessing familiar and novel food items. We hypothesized that because both have similar diets in this system, there would be little difference in their food preferences.

## METHODS

We conducted this study in Monteverde, Costa Rica, a montane cloud forest 1,500 meters above sea level. All animals we tested were found within a 1 km radius of the Monteverde Biological Research Station. This station keeps two sizeable compost piles in the nearby forest, where we located test subjects and ran most of our trials.

To test whether coatis and agoutis altered their foraging behavior when given a familiar-looking food item with a novel scent, we sliced pears into small cubes. We chose pears due to their relatively mild scent and because most of our study animals had probably encountered pears in compost before. To create two distinct scents, familiar and novel, we marinated the pear cubes in 400 ml of two separate scent cocktails. The first was the familiar scent, which consisted of a store-bought mixed juice containing mango, pineapple and orange juice. This constituted a familiar scent to coatis and agoutis alike, as these fruits appear frequently in the local compost piles. The second cocktail was the novel scent, which we created by mixing approximately 10 drops of wintergreen and clove oils diluted in water. We marinated the pear cubes for 4 hours prior to trials.

For each encounter with a coati or an agouti, we followed a consistent procedure. When we saw an individual of either species or suspected one to be in the area, we set out 4 pear cubes of each scent in two side-by-side squares, randomly alternating which scent we placed on the left or the right (Fig. 1). We also spread out several mango pieces to attract an individual to the area. We then backed away approximately 10 meters so our presence would not frighten the animal. Once an individual came within a meter of the test, we began filming the encounter, narrating which scented fruit the individual ate

first and how many of each scented fruit they consumed. Once the animal left the test and did not return for 5 minutes, we classified the encounter as complete.



Figure 1. Coati test subject entering experimental setup. In this case, cubes of pear with novel scent are on the right, and with familiar scent on the left.

## Statistical analysis

We analyzed the proportion of the first type of fruit consumed using a chi-square goodness-of-fit test under the null hypothesis that the experimental scents had no effect on consumption. We additionally quantified the willingness of each species to consume novel-scented fruit by calculating the proportion of individuals per species that consumed at least one piece of novel fruit during a trial and compared the proportions between species with a chi-square goodness-of-fit test. We used a 2-way contingency table analysis to evaluate whether willingness to try novel-scented fruit differed between agoutis and coatis.

## RESULTS

Both coatis and agoutis strongly preferred eating familiar-scented fruit over novel-scented fruit, but agoutis were slightly more willing to try novel-scented fruit than coatis (Fig. 2). Out of 42 coatis, all consumed familiar fruit first (chi-square = 42,  $p < 0.01$ ,  $df = 1$ ), and out of 8 agoutis, all but 1 individual consumed familiar fruit first (chi-square = 4.5,  $p < 0.05$ ,  $df = 1$ ).

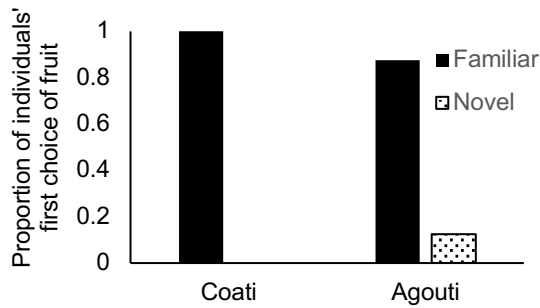


Figure 2. First type of fruit consumed by animal when presented with familiar and novel-scented fruit options.

Coatis and agoutis differed significantly in their willingness to try novel-scented fruit (Fig. 3; chi-square = 3.92,  $p = 0.02$ ,  $df = 1$ ). Each of the 3 coatis that tried novel fruit consumed less than 1 piece each, in many cases tasting a piece and spitting it out. On the other hand, 2 of the 3 agoutis that tried novel fruit consumed 3 of 4 novel fruit cubes and all familiar fruit.

#### DISCUSSION

The results of our study indicate that coatis and agoutis in this system are highly food neophobic. These results are consistent with our primary hypothesis that coatis and agoutis in this system should exhibit food neophobia as they are well-fed by local compost piles and, therefore, the cost of refusing a food item is low, while the cost of consuming a potentially harmful food item remains quite high. This simple cost-benefit analysis logically leads to the rejection of an unfamiliar food item in an environment where food scarcity is not an issue. The alternative hypothesis would predict both species to exhibit willingness to try novel-scented foods due to exposure to many novel foods in these compost piles; however, the highly neophobic tendencies we observed are inconsistent with this hypothesis.

While we can generally classify both species as food neophobic, there were significant differences between species in willingness to try novel-scented fruit. One potential explanation is the social dynamics of each species. Coatis are largely social animals, with females and young living in bands of up to forty individuals

(Gompper 1996). In one of our sampling locations, we observed several instances in which a coati consumed all familiar-scented fruit and left behind all novel-scented fruit before walking away. Shortly after, individuals in the area investigated the remaining novel-scented fruit and rejected it in the same manner. These observed behaviors align with the “costly information hypothesis” which suggests that individuals should rely on their own experiences to inform low-risk decisions, but when costs rise (e.g., a novel food item could be lethal) individuals should rely on social information rather than experimenting for themselves (Webster and Laland 2008). Conversely, agoutis are not very social animals. Therefore, our results support the hypothesis that social living can influence individual decision-making regarding novel food consumption. Additionally, these species exhibit different foraging behaviors. Coatis immediately eat objects they decide are food, whereas agoutis store food in their cheek pouches for later consumption. During trials, we assumed that the agoutis’ hoarding behavior indicated intentions of eating the fruit at a later point, but we do not know if there is an additional screening process of food prior to consumption.

The results of our study indicate a clear presence of food neophobia in both coatis and agoutis in the Monteverde system, with agoutis displaying a higher willingness to consume novel foods than coatis. In our study system, both species enjoy high food availability associated with living near human compost dumps, which may allow these animals the luxury of avoiding unnecessary dietary risks. Individuals of both species apparently acquire and evaluate sensory information when making decisions regarding what to eat and what not to eat. This information may come in the form of individual sensory cues (e.g., olfaction) or the behavior of conspecifics. Implications of this study are not limited to the Monteverde system, as understanding food neophobia and foraging patterns of scavenging animals may contribute to pest-management solutions and foster a greater understanding of behaviors of wild animals influenced by human activities.



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#### AUTHOR CONTRIBUTIONS

Both authors contributed equally.

#### LITERATURE CITED

- Alves-Costa, C. P., Da Fonseca, G. A., & Christófaró, C. (2004). Variation in the diet of the brown-nosed coati (*Nasua nasua*) in southeastern Brazil. *Journal of Mammalogy*, 85(3), 478-482.
- Gompper, M. E. (1996). Sociality and asociality in white-nosed coatis (*Nasua narica*): foraging costs and benefits. *Behavioral Ecology*, 7(3), 254-263.
- Henry, O. (1999). Frugivory and the importance of seeds in the diet of the orange-rumped agouti (*Dasyprocta leporina*) in French Guiana. *Journal of Tropical Ecology*, 15(3), 291-300.
- Launchbaugh, K. L., Provenza, F. D., & Werkmeister, M. J. (1997). Overcoming food neophobia in domestic ruminants through addition of a familiar flavor and repeated exposure to novel foods. *Applied Animal Behaviour Science*, 54(4), 327-334.
- Mitchell, D. (1976). Experiments on neophobia in wild and laboratory rats: a reevaluation. *Journal of Comparative and Physiological Psychology*, 90(2), 190.
- Rozin, P. (1968). Specific aversions and neophobia resulting from vitamin deficiency or poisoning in half-wild and domestic rats. *Journal of Comparative and Physiological Psychology*, 66(1), 82-88.
- Rozin, P. (1976). The selection of foods by rats, humans, and other animals. *Advances in the Study of Behavior*, 6, 21-76.
- Webster, M. M., and K. N. Laland. (2008). Social learning strategies and predation risk: minnows copy only when using private information would be costly. *Proceedings of the Royal Society of London B: Biological Sciences*, 275(1653), 2869-76.

## TROPICAL EPIPHYTE COMMUNITY STRUCTURE: NICHE PARTITIONING, ISLAND BIOGEOGRAPHY, AND SUCCESSION

ALEC COBBAN, ALEXANDER W. COTNOIR, CORINNE VIETORISZ, BALTHASAR VON HOYNINGEN HUENE

Teaching Assistants: Michael B. Brown and Deb Goedert  
Faculty Editor: Mark Laidre

*Abstract:* Despite their importance in contributing to the biodiversity, primary productivity, and biomass of tropical ecosystems, the factors structuring community composition of epiphytes remain unresolved. We examined factors that might influence epiphyte species richness and relative abundance in a neotropical cloud forest at Monteverde Biological Station, Costa Rica. We found evidence that relative abundances of mosses, lichens, and vines varied among trees with bark that was textured, smooth, or peeling. Within trees of similar bark morphology, epiphyte morphotype richness increased with tree circumference as a saturation function. Apparently, this epiphyte community is structured in part by niche partitioning, wherein epiphyte groups are adapted to thrive on different bark types according to their abilities to colonize, attach, and live in moist environments on bark surfaces. Furthermore, morphotype richness of epiphytes was related to tree size, which is consistent with the time-area hypothesis and island biogeography theory.

*Key words:* biodiversity, epiphytes, island biogeography, niche partitioning

### INTRODUCTION

Many ecological theories seek to explain what factors determine community composition and diversity. Possibilities include specialization, habitat area and age, and successional dynamics. One relevant theory pertains to niche partitioning. Given variation in available resources, niche partitioning theory suggests that coexisting species will be selected to specialize on different resources and therefore avoid competition. Other theories emphasize geographical area as determinants of community composition. Island biogeography theory predicts that overall diversity should increase in isolated communities as available area increases (MacArthur and Wilson 1967).

Other hypotheses add a temporal element: areas with longer geographic histories may support higher species richness, given more time for speciation and colonization (Fine 2015). Alternately, succession theory suggests that diversity tends to increase initially with colonization, and then decline over time due to competitive dominance. With enough time, a community may become dominated by relatively few abundant late-successional species, which outcompete colonizers and early-successional species. Succession can also change species composition without sweeping changes to

diversity. For example, two analogous communities in different successional stages may exhibit similar levels of diversity, despite being composed of different sets of species.

These theories can relate to community composition even at small scales. In this study, we examined communities of arboreal epiphytes at Monteverde, Costa Rica. The high-elevation cloud forest at this site supports rich communities of epiphytic lichens, mosses, vines, and bromeliads. Frequent surface-level cloud cover enriches the opportunities for these epiphytes to flourish.

Based on the above theory, we hypothesized that habitat characteristics would influence epiphyte community composition. Niche partitioning theory predicts some level of specialization among cloud forest epiphytes, with different epiphytes exploiting different microhabitats. For example, different trees with different bark morphology offer different substrates based on texture and stability of the bark surface. If so, occurrence of different epiphytes (e.g., vines, mosses, and lichens) should vary according to bark morphology.

Additionally, each tree constitutes a *de facto* island, consisting of a clearly-defined habitat separated from others by an environment unsuitable for epiphytes. Any epiphyte must

disperse through that environment to colonize another tree. Following island biogeography theory, larger trees may support higher epiphyte diversity, with fewer extirpations from disturbances. Assuming tree size is a proxy for tree age, larger trees may also carry higher epiphyte diversity based on the time-area hypothesis. Some epiphytes may not have had time to colonize a younger tree, or may need early colonist species to create a suitable surface. However, epiphyte diversity on large, old trees may be depressed by competitive dominance, and existing studies have found varying correlations between tree size and epiphyte diversity (Dislich and Mantovani 2016, Hietz and Hietz-Seifert 1995). Thus, epiphyte diversity could increase with tree size (and therefore age), or there could be reduced diversity on the largest trees due to competitive dominance of relatively few late-successional species.

## METHODS

### *Sampling Site*

In this study, we examined communities of arboreal epiphytes surrounding Monteverde Biological Station, situated in the tropical Monteverde cloud forest in Costa Rica. The main features characterizing Montverde's climate include relatively cool montane temperatures (9-23 degrees Celsius), heavy seasonal rainfall and fog (mean annual precipitation = 1460 mm), and the influence of strong northeast trade winds. The vegetation of Monteverde is characterized by an impressive abundance of epiphytes, including 81 species of herbaceous and semi-woody vines, and 878 species of lichens, mosses, and bromeliads (Nadkarni and Wheelwright 2000). The distance between sampling sites in this study was deliberately small to minimize variation in precipitation, altitude, and temperature that are also known to affect epiphyte distribution.

### *Bark Morphology*

To test whether epiphyte communities are structured in part by niche partitioning across different bark morphologies, we first identified three distinct bark types: smooth, textured, and peeling (Figure 1). Trees with peeling bark shed strips of bark on a regular basis, whereas textured

bark trees possess a spongy, thick, rippled surface with large vertical cracks. Smooth trees provided an intermediate bark type, having a smooth surface that does not shed regularly. We selected 15 trees for comparison (5 of each bark morphology) while walking two forest trails extending south and west from Monteverde Biological Station, no further than 2 km from the station and all at similar elevation. All study trees were ~100 cm circumference growing under similar light conditions.



Figure 1. Photos showing the categorization of bark morphologies. From left to right: peeling, textured, and smooth (non-peeling) bark.

At all 15 trees, we measured trunk circumference 1 meter from the ground, and delineated a belt transect circling around the trunk at 1 – 1.2 meters from the ground. Within this transect on each tree, we estimated species richness of vines, mosses, and lichens by counting the number of morphotypes within each epiphyte category.

### *Tree Circumference*

We selected another 30 trees within the same study area, but in this case varied tree circumference while holding bark morphology constant (smooth). We were unable to keep tree species constant, but randomized our tree selection within the constraints of bark morphology. Sample trees were separated from each other by a random distance between 1-15 meters.

### *Species cover analysis*

At each tree, we placed one square frame of 3, 5, 10, or 20 cm x 20 cm per side against the north and south face of the tree trunk at 1 meter from the ground. We selected frame size based on the tree being sampled needed to encapsulate about three-quarters of the diameter of the tree. We took

digital photographs of the framed north and south faces of the trunk, and then quantified the relative coverage of mosses and lichens as a proportion of total area using ImageJ software (Figure 2).

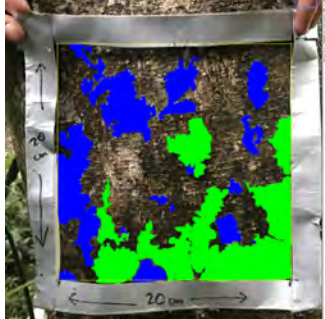


Figure 2. Process of categorizing percent moss (green) and percent lichen (blue) cover using ImageJ software. Percentage moss and percentage lichen were each calculated as the number of pixels out of the entire number of pixels inside the frame area.

#### Canopy cover analysis

To evaluate patterns in epiphytes related to light availability, we captured a photograph of the canopy directly overhead at each tree. Canopy cover pictures were imported to ImageJ software and converted to binary colors. Percent open canopy was then calculated using the histogram feature.

#### Statistical methods

We performed all statistical analyses in JMP Pro 13.0. Tree circumference data was log transformed to meet assumptions of normality. We performed one-way ANOVA tests for moss coverage, lichen coverage, and number of vines for each of the different bark types.

To model the rate at which epiphyte species accumulate based on tree surface area, we fit a non-linear model to our data (eq. 1), using a function derived from a photosynthetic rate model. The parameters were fit using an Analytic Gauss-Newton algorithm.

$$\frac{b \times \text{total area}(\text{cm}^2)}{1 + a \times \text{total area}(\text{cm}^2)} - R$$

Equation 1.  $a$  = the initial slope of the species accumulation curve,  $b$  = the inverse of the total species at species saturation, and  $R$  = the y-intercept.

#### RESULTS

Bark morphology was related to abundances of mosses, lichens, and vines (Figure 3;  $F_{2,12} = 6.13$ ,  $P = 0.02$ ). Post hoc contrasts indicate that there was no difference in moss cover between smooth and textured bark ( $t = 0.66$ ,  $P = 0.52$ ,  $df = 12$ ), but moss cover was greater in both smooth ( $t = 3.31$ ,  $P = 0.006$ ,  $df = 12$ ) and textured bark compared to peeling bark ( $t = 2.65$ ,  $P = 0.02$ ,  $df = 12$ ). A one-way ANOVA shows that lichen cover varied across bark types ( $F_{2,12} = 22.8$ ,  $P < 0.0001$ ). Post hoc contrasts indicated that lichen cover was significantly greater on smooth bark compared to peeling ( $t = 3.80$ ,  $P = 0.003$ ,  $df = 12$ ) and textured bark ( $t = 6.74$ ,  $P < 0.0001$ ,  $df = 12$ ), and lichen cover was significantly greater on peeling bark compared to textured bark ( $t = 2.95$ ,  $P = 0.01$ ,  $df = 12$ ). Vine abundance also varied with bark type ( $F_{2,12} = 14.3$ ,  $P = 0.0007$ ): more vines on textured bark than either smooth ( $t = 4.43$ ,  $P = 0.0008$ ,  $df = 12$ ) or peeling bark ( $t = 4.82$ ,  $P = 0.0004$ ,  $df = 12$ ). Vine abundance did not differ between peeling and smooth trees (Figure 3;  $t = 0.39$ ,  $P = 0.70$ ,  $df = 12$ ).

As host tree surface area increased, the total number of epiphyte morphotypes increased rapidly and then began to saturate (Figure 4).

Lichen abundance (percent lichen) decreased as tree circumference increased. Moss abundance (percent moss) showed no relation with tree size (Figure 5; slope  $\pm$  SE =  $3.92 \pm 4.58$ ,  $P = 0.40$ ,  $r^2 = 0.03$ ).

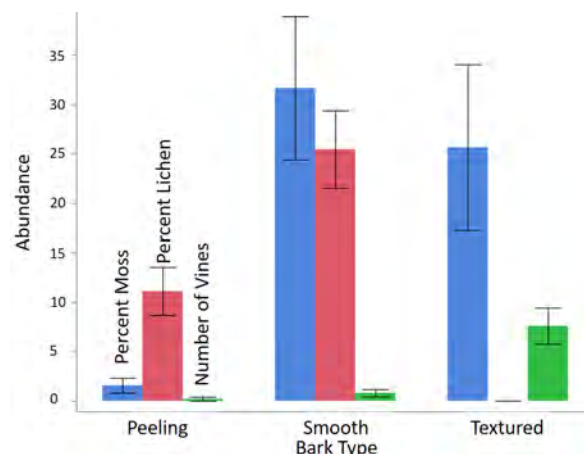


Figure 3. Relative abundance of moss, lichen, and vines by tree bark type. Moss and lichens expressed as percent area; vines expressed as counts. Error bars represent one standard error from the mean.

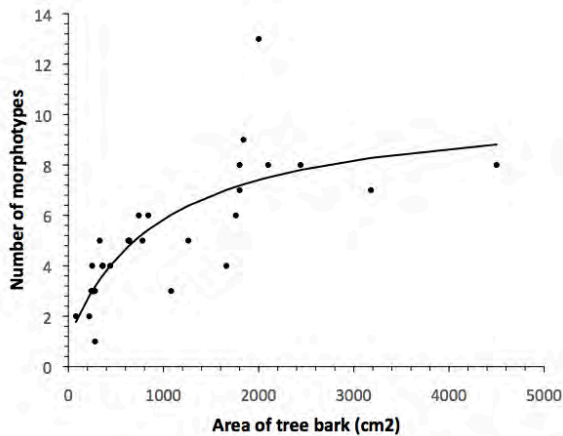


Figure 4. Saturation curve of number of epiphytes by surface area. Parameter estimates for equation 2 were:  $a \pm SE = 0.001 \pm 0.0009$ ,  $b = 0.010 \pm 0.008$ ,  $R = -1.06 \pm 1.48$ .

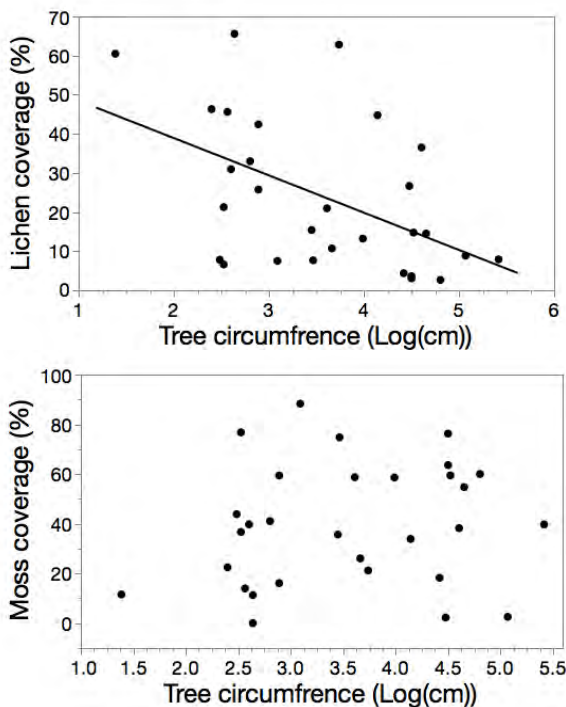


Figure 5. Percent coverage by lichen (upper) and moss (lower) versus tree circumference. Each point represents the average coverage of 2 plots from each of 30 study trees sampled.

## DISCUSSION

The epiphyte communities we studied were related to both bark morphology and tree size. Patterns with respect to bark types (peeling, smooth, and textured) indicate that niche partitioning is a factor in the assembly of

lichens, mosses, and vines. In accord with niche theory, environmental variables (in this case, host tree bark structure) appear to structure communities based on differential adaptations to variable ecological conditions (Dislich & Mantovani 2016). Key adaptations may include capacity to cling to bark surfaces, tolerate differences in moisture availability, and colonize new host trees. Our results are consistent with previous findings that vines exhibit strong host preferences for specific tree species (Burns 2005). Our data also suggest that textured bark provides better environmental conditions for moss, perhaps because mosses thrive in cool, moist environments, which are provided in the crevices of textured bark (Sales et al. 2016). Our data are consistent with the idea that mosses are competitively dominant over lichen on bark types with plentiful shaded regions (Rainus et al. 2008). Overall, highly textured bark provides shelter from direct sun, increased humidity, and attachment security, all factors which favor occupancy by mosses (Sales et al. 2016).

On the other hand, lichens seemed better able to colonize peeling bark, perhaps because they are efficient colonizers and can withstand drier conditions relative to moss (Armstrong and Welch 2007). Co-occurrence of moss and lichens was greatest on smooth non-peeling bark, which is intermediate in respect to age and moisture.

Following the tenets of island biogeography theory, there was a positive relationship between tree size and epiphyte diversity. Our findings match those of epiphytes in a Mexican cloud forest (Hietz and Hietz-Seifert 1995) but conflict with patterns between tree size and epiphyte richness reported for a Brazilian lowland forest (Dislich and Mantovani 2016). These differences may correspond to environmental differences between cloud and lowland forests or may appear because epiphyte communities on older trees have reached an equilibrium state/climax community. If we consider tree host size as a proxy for tree age, a larger host has been exposed to epiphyte seed rain longer and has a larger surface area for colonization and an elevated epiphytic habitat with a larger range of abiotic conditions (MacArthur and Wilson 1967). In our study, the total number of species approximated by a saturation curve as total area of the belt

transect increased. The saturation may arise because, after a certain size is reached, there are no more available niches, all the present resources have been exploited, or the pool of potential species has been fully exhausted.

Some interpretations from our study are limited because we were unable to control for forest successional stage (Barthlott et al. 2001) or variance in growth rate across the tree species we sampled. We were also unable to directly observe the mechanisms by which lichen and moss distribute themselves across individual bark surfaces. Future studies could evaluate community composition across finer scales, such as shaded and non-shaded microhabitats

Our results provide insights into the forces structuring epiphyte communities by showing that epiphytes differentially occupy different bark morphologies, and that tree host size corresponds to epiphyte species diversity. Community composition influences higher-level ecosystem processes such as productivity, stability, and susceptibility to invasion (Tilman 1999); therefore, a mechanistic understanding of how communities are assembled can reveal how ecosystems function and inform conservation efforts. Understanding the drivers of epiphyte distribution within tropical ecosystems can help forestry management maximize diversity and protect these unique habitats within a rapidly changing world.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

Barthlott, W., Schmit-Neuerburg, V., Nieder, J., and S. Engwald. 2001. Diversity and abundance of

vascular epiphytes: a comparison of secondary vegetation and primary montane rain forest in the Venezuelan Andes. *Plant Ecol* 152:145–156.

Burns, K.C. 2005. Patterns in the diversity and distribution of epiphytes and vines in a New Zealand forest. *Austral Ecology* 30:883-891.

Dislich, R., and W. Mantovani. 2016. Vascular epiphyte assemblages in a Brazilian Atlantic forest fragment: Investigating the effect of host tree features. *Plant Ecology* 217:1-12.

Fine, P. V. A. 2015. Ecological and evolutionary drivers of geographic variation in species diversity. *Annual Review of Ecology, Evolution, and Systematics* 46:369-92. doi: 10.1146/annurev-ecolsys-112414-054102

Hietz, P., and U. Hietz-Seifert. 1995. Structure and ecology of epiphyte communities of a cloud forest in central Veracruz, Mexico. *Journal of Vegetation Science* 6:719-728.

MacArthur, R. H., and E. O. Wilson. 1967. *The Theory of Island Biogeography*. Princeton, NJ: Princeton University Press.

Munoz, A. A., Chacon, P., Perez, F., Barnert, S. E., and Armesto, J. J. 2003. Diversity and host tree preferences of vascular epiphytes and vines in temperate rainforest in southern Chile. *Australian Journal of Botany* 51:381-391.

Nadkarni, N. M. 2000. Colonization of stripped branch surfaces by epiphytes in a lower montane cloud forest, Monteverde, Costa Rica. *Biotropica* 32:358-363.

Nadkarni, N. M. and Wheelwright, N. T. 2000. *Ecology and Conservation of a Tropical Cloud Forest*. New York, NY: Oxford University Press.

Ranius, T., Johansson, P., Berg, N et al. (2008) The influence of tree age and microhabitat quality on the occurrence of crustose lichens associated with old oaks. *Journal of Vegetation Science* 19:653-662.

Sales, K., Laurie, K., and Gardner, J. Factors influencing epiphytic moss and lichen distribution within Killarney National Park. *Bioscience* 9:1-13.

Silvertown, J. 2004. Plant coexistence and the niche. *Trends in Ecology & Evolution* 19:605-611.

Tilman, D. 1999. The Ecological consequences of changes in biodiversity: a search for general principles. *Ecology*. 80:1455-1474.



## BIOTIC AND ABIOTIC DETERMINANTS OF REPRODUCTIVE POTENTIAL IN AN UNDERSTORY FERN (PTERIDOPHYTA)

CLAYTON E. JACQUES

Teaching Assistants: Michael Butler Brown and Debora Goedert

Faculty Editors: Matthew P. Ayres and Mark E. Laidre

**Abstract:** Reproduction is a central feature in the life history of all organisms, but investment in reproduction is subject to trade-offs from alternative uses for resources. Reproduction by individuals can vary with abiotic and biotic factors. I tested the effect of sunlight availability (abiotic) and local conspecific density (biotic) on the reproductive potential of hammock ferns *Blechnum occidentale*. The proportion of fertile fronds (with sori) was higher in plants with relatively open canopies that permitted more sunlight to reach the ferns. In contrast, higher local densities of ferns were unrelated to reproduction, perhaps because adjacent fern ramets were frequently connected below ground and apparently part of larger clones.

**Key words:** canopy cover, competition, density, hammock fern, reproductive potential

### INTRODUCTION

Investment in reproduction usually has trade-offs with other organismal functions. For instance, producing reproductive structures can increase offspring; however, the parent shoulders energetic and opportunity costs. Among plants, reproduction can incur penalties in growth, maintenance, defenses, and future reproductive potential (Obeso 2002). Local conditions can influence resource allocation. Due to repercussions, organisms modify reproductive investment according to biotic and abiotic factors – including resource availability and conspecific competition.

Numerous ecological studies have showed the necessity of environmental resources for plant reproduction. Abiotic factors important for reproduction may include nutrients, moisture, and sunlight. Bazzaz et al. (1987) describe a general relationship among species between increasing resource availability, increasing conversion of resources into biomass, and increasing allocation to plant reproduction. These relationships may vary among individuals within a species, depending upon size, morphology, and resource availability.

High local density of conspecifics may induce competition for limiting resources. Due to limited dispersal capabilities, individual plants often coexist with conspecifics or closely related kin (Simonsen *et al.* 2014). Local density of conspecifics functions as a biotic factor

affecting reproductive potential. He and Bazzaz (2003) observed that increasing conspecific density reduces individual plant biomass and reproductive productivity among American pokeweed (*Phytolacca americana*). However, the effect of local density may differ according to species needs and availability of resources.

My study investigated how local biotic and abiotic conditions influence reproduction in a species of understory fern, probably the hammock fern *Blechnum occidentale*. Understanding how such factors influence reproduction in the hammock fern will inform theories of life history trade-offs. A common Central American plant, the hammock fern grows in clusters under partial shade (Bigelow and Kukle 1991). Sterile and fertile fronds are slightly dimorphic; the fern is easily identified by a diagnostic swath of spore-producing sori along the midrib of fertile pinnae. I hypothesized that sunlight is a limiting factor for the hammock fern's reproduction and predicted that greater open canopy would increase reproduction. I further hypothesized that conspecific ferns compete for resources, which predicts that greater local density of hammock ferns would reduce reproductive potential.

### METHODS

#### *Field Observations*

In January 2018, I demarcated 21 plots of 0.5 x 0.5 m near the Estación Biológica Monteverde,



Costa Rica. Each plot contained ramets of *Blechnum occidentale*. I estimated the percentage of open canopy over each plot as the average of four measurements with a spherical densiometer. Within each plot, I removed the leaf litter and counted the number of heterospecific plants, number of hammock fern ramets, total number of living fronds per ramet, and number of fertile fronds (with dark, visible sori on leaflet midrib). Following Durand and Goldstein (2001), I used the fertility of fern fronds as a measure of reproduction. I did not count single fronds to exclude ramets that were not reproductively mature. I excavated the top layer of soil from ten of the plots to view fern root structures and look for below-ground connections between ramets, an indicator of clonal relationship.

#### Statistical Analyses

I calculated the proportion of fertile fronds per ramet by dividing the number of fertile fronds by the total number of living fronds. I calculated the mean of this proportion for all ramets at each plot. To improve normality for statistical analyses, I performed a natural-log transformation (+ 0.1 to eliminate zeros) of the mean proportion of fertile fronds. To evaluate the effect of sunlight on reproductive potential, I conducted a least squares linear regression analysis on log-transformed mean proportion of fertile fronds per plot as a function of percentage open canopy. To investigate the effect of local density on reproductive potential, I also conducted a least squares regression analysis for the mean proportion of fertile fronds by the number of ramets per plot. All data analyses were performed in JMP Pro 13.0.

#### RESULTS

The number of ferns per plot ranged from 2 to 23 (mean  $\pm$  SD =  $10.8 \pm 6.3$ ). The number of fronds per fern ranged from 2 to 14 (mean  $\pm$  SD =  $3.9 \pm 2.1$ ). Canopy cover was inversely related to the proportion of fertile fronds per plot (Figure 1; slope  $\pm$  SE =  $0.081 \pm 0.03$ ,  $P = 0.028$ ,  $R^2 = 0.23$ ). The number of fern ramets was unrelated to the proportion of fertile fronds per plot (Figure 2; slope  $\pm$  SE =  $-0.0136 \pm 0.0076$ ,  $P = 0.09$ ,  $R^2 = 0.14$ ). I performed the same

analysis with the natural-log transformed data; the results were also not significant.

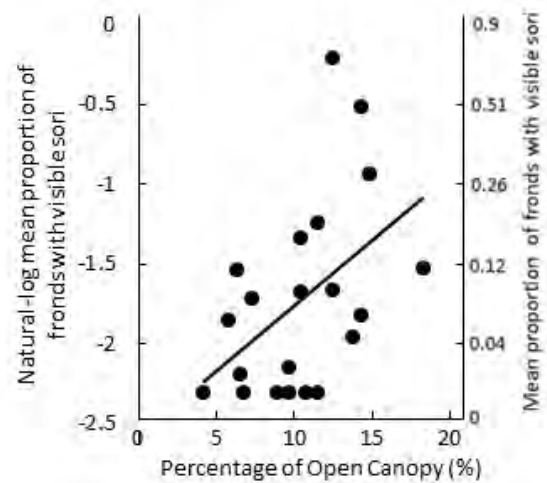


Figure 1. Mean proportion of hammock fern fronds with visible sori increased as a function of the percentage of open canopy (%). Least squares regression line displayed.

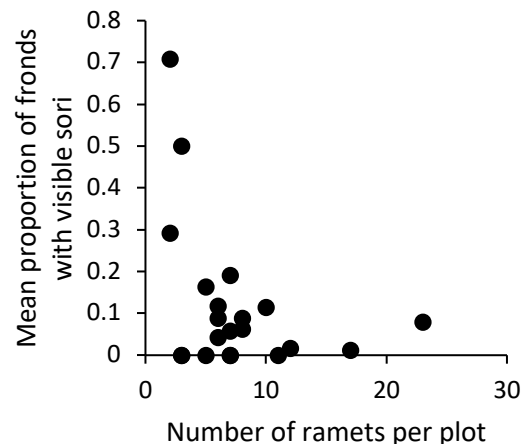


Figure 2. The proportion of hammock fern fronds with visible sori was unrelated to the number of fern ramets per plot.

Plot excavations indicated that some hammock fern ramets were the same clone (same genet). I discovered root connectivity in five of the ten plots investigated (50%). Four instances involved root linkages between two ramets with sterile fronds. One instance involved root linkages between three ramets, two with sterile fronds and a third with one fertile frond.

## DISCUSSION

The reproductive potential of the hammock fern varied with canopy cover but not local density. Sunlight exposure (measured as the percentage of open canopy) appears to be a limiting abiotic resource for fern reproduction (Figure 1), consistent with my initial hypothesis. This finding is consistent with broad ecological resource allocation theory among plants – plants with more resources can invest more in reproduction (Bazzaz *et al.* 1987). Further studies could investigate if photosynthetically active area (measured as leaf area) predicts the reproductive potential of individual ramets.

There was no support for the hypothesis that local density of hammock ferns leads to increased competition for resources (e.g. sunlight) which would reduce investment in reproduction. However, as fern density increased, the mean reproductive potential of ramets did not vary in a significant way (Figure 2). Interconnectivity between ramets provides a possible explanation for this trend. Some genets within my sample plots produced multiple ramets, which could share resources or chemical signals via root connections. Murphy and Dudley (2009) found that *Impatiens cf. pallida* can distinguish related and stranger plants and adjust structural growth to avoid competition with kin; these plants invest in shoot growth to overshadow unrelated competitors but increase root growth in the presence of relatives. Hammock ferns might also adjust root-shoot and reproductive-sterile tissue investment ratios with the degree of relatedness and physiological connectivity. Availability of abiotic resources often affects plant reproduction, yet conspecific density may not always yield competition for resources that reduces reproduction – especially in clonal plants where genets are bigger than ramets.

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## LITERATURE CITED

- Bazzaz FA, Chiariello NR, Coley PD, and Pitelka LF. 1987. Allocating resources to reproduction and defense. *Bioscience* 37: 58–67.
- Bigelow S and Kukle P. 1991. *Blechnum occidentale*. In: The Ferns of Monteverde. San José, Costa Rica: Tropical Science Center and Marie Selby Botanical Gardens.
- Durand LZ and Goldstein G. 2001. Growth, leaf characteristics, and spore production in native and invasive tree ferns in Hawaii. *Am Fern J* 91: 25–35.
- He JS and Bazzaz FA. 2003. Density-dependent responses of reproductive allocation to elevated atmospheric CO<sub>2</sub> in *Phytolacca americana*. *New Phytol* 157: 229–39.
- Murphy GP and Dudley SA. 2009. Kin recognition: Competition and cooperation in *Impatiens* (Balsaminaceae). *Am J Bot* 96: 1990–6.
- Obeso JR. 2002. The costs of reproduction in plants. *New Phytol* 155: 321–48.
- Simonsen AK, Chow T, and Stinchcombe JR. 2014. Reduced plant competition among kin can be explained by Jensen's inequality. *Ecol Evol* 4: 4454–66.

## EFFECTS OF WEATHER, BODY SIZE, AND BEHAVIORAL SEQUENCES ON HUMMINGBIRD FORAGING

HANNAH I. HOFFMAN AND MAANAV JALAN

Teaching Assistants: Michael Butler Brown and Debora Goedert

Faculty Editors: Matthew P. Ayres and Mark E. Laidre

**Abstract:** Behavior is shaped by both context-specific and individual factors such that organisms draw from wide behavioral repertoires to maximize their fitness in various environments. This concept may be particularly useful in understanding foraging ecology, which explores how animals reach physiologically-determined nutritional and energetic requirements while balancing fitness costs. Hummingbirds have the highest metabolic rate of any vertebrate and must thus ingest a near-constant supply of calories; therefore, these species emerge as model organisms in which to study foraging, as they likely employ tactics well-tuned to their environments. We aimed to evaluate context-specific and individual factors that influence hummingbird foraging rates. Foraging rates could be negatively correlated with high temperatures due to thermoregulatory needs, with high rainfall due to flight inhibition, and with low light intensity due to visibility. Metabolic theory, which states that smaller species have higher mass-specific metabolism suggests that foraging rates should be greater in smaller hummingbird species than in larger ones. We analyzed foraging bouts of three hummingbird species (*Eupherusa eximia*, *Elvira cupreiceps*, and *Colibri thalassinus*) under variable weather in Monteverde, Costa Rica. We also analyzed foraging, perching, and territoriality for a single hummingbird (*Eupherusa eximia*). The three abiotic factors were unrelated to foraging rates. Foraging rates were slower after bouts of defense than after periods of perching, suggesting that hummingbirds incur a cost to foraging from territorial behavior.

**Key words:** Hummingbirds, foraging behavior, perching behavior, territory defense

### INTRODUCTION

Behavior is shaped by both context-specific and individual factors such that organisms draw from behavioral repertoires to maximize their fitness in various environments. This concept may be particularly useful in understanding foraging ecology, which explores how animals reach physiologically-determined nutritional and energetic requirements while balancing fitness costs such as exposure to severe weather and territory defense. Efficient foragers use their behavioral repertoire to optimize caloric intake given their physiology and abiotic environment.

Hummingbirds present a particularly suitable system in which to study foraging behavior, as they have the highest mass-specific metabolic rates among vertebrates and need a near-constant supply of flower nectar during the day (Gass et al. 1999). This high caloric requirement necessitates efficient foraging that maximizes intake under the pressures of shifting abiotic conditions and biotic interactions.

Numerous studies discuss the influences of abiotic factors on hummingbird foraging behavior. Hummingbirds are known to forage

with higher rates of energy intake in colder conditions as a thermoregulatory strategy (Gass et al. 1999). In heavy rain, hummingbirds alter their wing speed and body orientation to account for hindrance from raindrops (Ortega-Jimenez et al. 2012). Lastly, Grubb (1977) has suggested that birds forage more efficiently with increasing light intensity due to improved vision.

Body size can also influence hummingbird foraging behavior. Mass-specific metabolic rates of animals generally increase with decreasing body mass. Therefore, smaller hummingbird species require more food and could be expected to feed longer or more often.

Hummingbird behavior includes not only feeding but also perching and defending territory. More time spent perching or defending territories could place limits on the time available for feeding.

We tested for patterns in hummingbird foraging rates in Monteverde, Costa Rica. Monteverde hosts over 30 hummingbird species and has famously variable weather (Monteverde Tours). We evaluated the effects of three abiotic factors – temperature, rain, and light intensity –

on foraging rate. We hypothesized that foraging rates should be higher in lower temperatures as thermoregulatory compensation, lower in rain as mechanical compensation, and higher in brighter light with increased visibility. In addition, we compared foraging efficiencies between species of hummingbirds, with metabolic theory predicting the highest foraging rates in the species with the smallest mass. We also tested for patterns in behavioral sequences. If perching is important for rest and recovery, (1) perch durations and subsequent activity durations should be positively correlated and (2) activity durations and subsequent perch durations should be positively correlated. Furthermore, foraging rates could differ depending on if they occur directly after perching or directly after defending territory. An increase in post-defense foraging rates would imply a need for more nectar after costly territorial displays, while a decrease in post-defense foraging rates would imply that territorial displays limit capacity for foraging immediately thereafter.

## METHODS

We filmed individual hummingbird foraging bouts, which spanned a bird's visit to a bush, for three different species of hummingbirds (Green Violetears (*Colibri thalassinus*), Stripe-tailed Hummingbirds (*Eupherusa eximia*), and Coppery-headed Emeralds (*Elvira cupreiceps*)) in Monteverde, Costa Rica. All videos were recorded on three bushes of the same plant species (Family: Verbenaceae). We controlled for flower variation in our observations by collecting data from only one species. Directly following a foraging bout, we recorded: (1) light intensity using the Pyranometer iPhone application by pointing the camera directly away from the bush at 90 degrees; (2) temperature by placing a thermometer at roughly the distance the hummingbird's body stayed from the bush; (3) rainfall recorded as presence or absence of rain, quantified as 1 and 2, respectively. We counted the number of bill insertions into flowers (dips) per second as a proxy for foraging rate by randomly selecting a 20-second period to analyze in a recorded bout.

To understand if and how weather influenced foraging rates, we evaluated a suite

of seven candidate linear regression models including all possible combinations of our three abiotic factors.

To determine whether species – and thus body size – influenced foraging rates, we conducted a two-sample t-test comparing the average foraging rates of Green Violetears and Stripe-tailed Hummingbirds. We excluded Coppery-headed Emeralds from this analysis due small sample size (N=3).

To investigate the effect of perching and territoriality on hummingbird foraging behavior, we collected continuous video footage of an individual Green Violetear (*Colibri thalassinus*) for forty-five minutes. We chose this hummingbird because it continuously returned to the same perch and stayed in visible range for an extended period of time. Through video analysis, we generated an activity log for the individual by timing each perching event and each period spent away from the perch. We examined frequency histograms of displaying perch durations and length of times spent away from perch. We used simple linear regressions to test for effects of perch duration on subsequent activity duration, and the second for the effect of activity duration on subsequent perch duration. Durations were log-transformed for statistical analyses, but figures show untransformed data.

Lastly, to examine whether territorial displays impacted foraging rates, we conducted a two-sample t-test comparing foraging rates immediately after defense (i.e., chasing another hummingbird) with rates immediately after perching. All analyses were conducted using JMP13 software.

## RESULTS

Temperature, rainfall, and light intensity did not influence foraging rates individually or in any possible combination ( $P>0.09$ ). Temperature ranged from 15.4 to 19.2 °C, light intensity ranged from 9 to 256 W/m<sup>2</sup>, and rainfall was present in 23 of 32 sampling occasions.

Green Violetears (*Colibri thalassinus*) were about 25% larger (5.25 vs 4.27 g; hbw.com, The Cornell Lab of Ornithology) and foraged about 20% more slowly than Stripe-tailed Hummingbirds (*Eupherusa eximia*) ( $t(18)=2.26$ ,  $P<0.05$ ) (Figure 1).

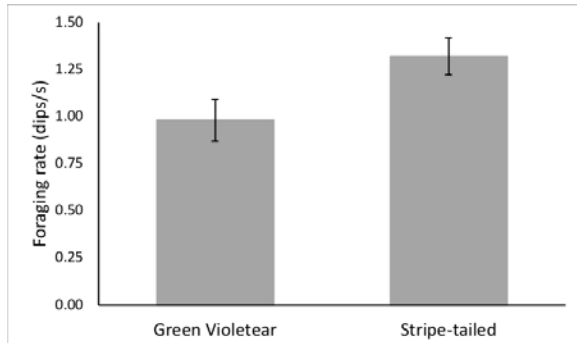


Figure 1. Average foraging rates (dips/s) of Green Violetears (*Colibri thalassinus*) and Stripe-tailed Hummingbirds (*Eupherusa eximia*). Standard error bars are displayed. Stripe-tailed Hummingbirds had a significantly higher average foraging rate than Green Violetears. (N=8 and 11, respectively)

The duration of perching for Green Violetear was typically 1 to 80 seconds but ranged as high as 240s, and durations away from its perch were typically less than 20 seconds (Figure 2).

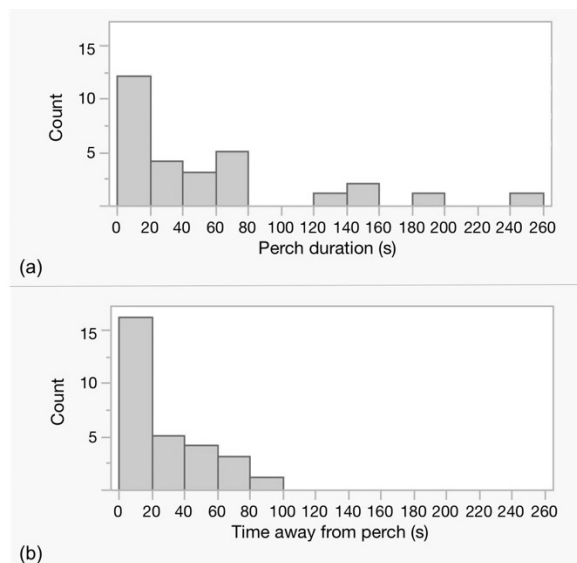


Figure 2. Distributions of (a) perch durations (N=29) and (b) lengths of times spent away from perch (N=29) for a Green Violetear hummingbird (*Colibri thalassinus*).

The amount of time the Green Violetear perched was unrelated to the time it subsequently spent away from its perch ( $F(1,23)=4.15$ ,  $P>0.05$ ). Additionally, the amount of time it spent away from its perch was unrelated to the time it subsequently perched ( $F(1,24)=0.26$ ,  $P>0.05$ ) (Figure 3).

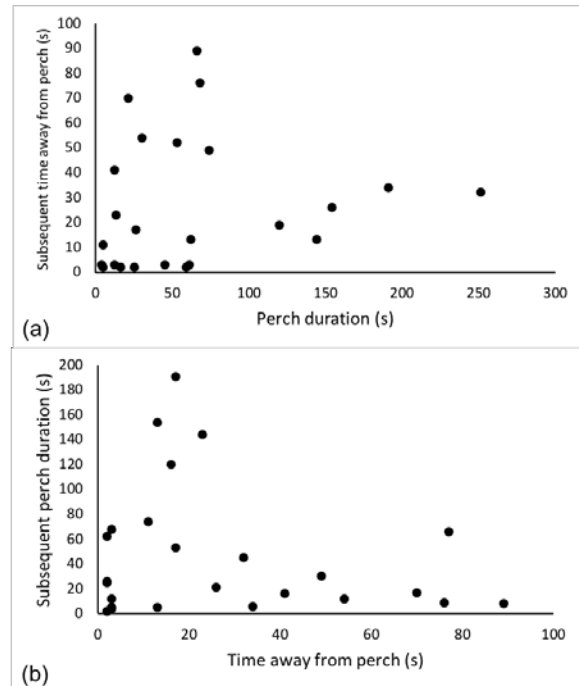


Figure 3. Relationships between (a) perch duration and subsequent time spent away from perch (N=24 events) and (b) time spent away from perch and subsequent perch duration (N=25 events) for a Green Violetear hummingbird (*Colibri thalassinus*). Neither relationship was significant ( $P>0.05$ ).

Average foraging rate after perching was significantly higher than average foraging rate after defending territory (mean $\pm$ SE=1.90 $\pm$ 0.10 vs 1.30 $\pm$ 0.22 dips/s) (t(17)=4.50, P<0.001) (Figure 4).

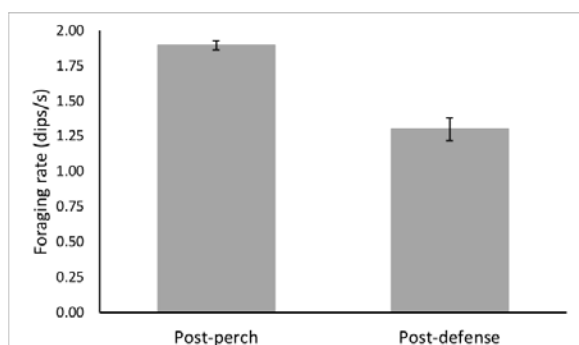


Figure 4. Average foraging rate (dips/s) for a Green Violetear hummingbird (*Colibri thalassinus*) were higher directly after perching (N=10) compared to directly after territorial defense (N=8). Standard error bars are displayed.

## DISCUSSION

Of the factors tested, body size and territoriality were related to hummingbird foraging rates. The smaller species foraged at a higher rate than the larger one, and, our focal hummingbird foraged at a higher rate after perching than after defending its territory.

The finding that abiotic factors did not influence foraging rates suggests that Monteverde hummingbirds do not respond to micro-variations in their environment, which is inconsistent with previous reports. While hummingbirds do not seem to thermoregulate by increasing their foraging rate in colder temperatures, perhaps they alter other behaviors such as wing amplitude or wing speed to increase body temperature (Altshuler et al. 2002). Rain droplets do not seem to weigh down these hummingbirds and thus decrease their foraging rates (Ortega-Jimenez et al. 2012). It is possible that hummingbird species in Monteverde are adapted to maintain similar foraging rates regardless of rain, as they might not consume enough calories if they decrease caloric intake in response to the very frequent rains. The lack of response to a large range of light intensities suggests that visibility does not influence foraging rates (Grubb 1975). Given that hummingbirds require a near-constant supply of food throughout the day, during which there is great variation in light intensity, slowing foraging in periods of low light could be disadvantageous.

The effect of body size on foraging rate supports metabolic theory, with the smaller hummingbird likely having a faster metabolic rate. Future studies could verify that the Stripe-tailed Hummingbird does indeed have a faster metabolic rate than the Green Violetear.

The fact that no relationships were found between perch durations and times spent away from the perch suggests that longer perch durations do not provide superior rest. While a hummingbird might need to perch for at least a certain duration after activity, rest does not appear to be the only purpose for this behavior. This study provides evidence for the possibility that hummingbirds perch periodically to ensure their territory, as the observed hummingbird appeared vigilant while perching, seemingly

surveying its territory with rapid head movements.

One explanation for the finding that the Green Violetear foraged significantly less efficiently if it had just chased another hummingbird from its territory than if it had just perched is that defending territory is energetically costly. In other words, a hummingbird might simply be too exhausted post-defense to maintain its post-perch dipping speeds. Another possibility is that post-defense foraging serves a different purpose than post-perch foraging; perhaps the hummingbird's sole motivation post-defense was not to consume nectar but to physically demonstrate control over a territory to the intruder by feeding. It is also possible that hummingbirds are distracted by intruders, leading to less focused and thus slower feeding.

The findings of this study might result from the use of foraging rate as a dependent variable rather than foraging success (i.e., caloric intake); therefore, all recorded dips might not have resulted in identical nectar extraction. Accounting for caloric intake might provide deeper understanding into the effects of abiotic and individual factors on hummingbird foraging. Similar research could be conducted with video equipment that can track nectar extracted per second.

In a species that relies on near-constant caloric intake, it comes as a surprise that foraging tactics are not fine-tuned to abiotic factors that could reduce energy efficiency. The most advantageous feeding behavior does not seem to account for the physical environment but rather depends upon individual and context-specific factors such as body mass, perching, and defending territory. Foraging behavior thus cannot be understood as responses to a list of gradients but rather as a part of an organism's energy and time budget.

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AUTHOR CONTRIBUTIONS

Both authors contributed equally.

LITERATURE CITED

- Altshuler, D. L., & Dudley, R. 2002. The ecological and evolutionary interface of hummingbird flight physiology. *Journal of Experimental Biology*, 205: 2325-2336.
- Coppery-headed Emerald *Elvira cupreiceps*. The Cornell Lab of Ornithology. Published online at <https://neotropical.birds.cornell.edu/Species-Account/nb/species/cohemel/appearance>, accessed 1/22/18 [Website]
- Gass, C. L., Romich, M. T., & Suarez, R. K. 1999. Energetics of hummingbird foraging at low ambient temperature. *Canadian Journal of Zoology*, 77: 314-320.
- Green Violet-ear (*Colibri thalassinus*). Hbw.com. Published online at <https://www.hbw.com/species/green-violet-ear-colibri-thalassinus>, accessed 1/22/18 [Website]
- Grubb, T. 1977. Weather-Dependent Foraging Behavior of Some Birds Wintering in a Deciduous Woodland: Horizontal Adjustments. *The Condor*, 79: 271-274
- Monteverde Orchids and Hummingbirds. Monteverdetours.com. Published online at <https://monteverdetours.com/monteverde-orchids-and-hummingbirds.html>, accessed 1/22/18 [Website]
- Ortega-Jimenez, V., & Dudley, R. 2012. Flying in the rain: Hovering performance of Anna's hummingbirds under varied precipitation. *Proceedings: Biological Sciences*, 279: 3996-4002.
- Stripe Tailed Hummingbird *Eupherusa eximia*. The Cornell Lab of Ornithology. Published online at <https://neotropical.birds.cornell.edu/Species-Account/nb/species/stthum1/appearance>, accessed 1/22/18 [Website]



## SOCIAL BUTTERFLIES: FISSION-FUSION DYNAMICS IN *MANATARIA MACULATA*

GRACE S. CALLAHAN, PAUL J. VICKERS, AND MARY M. PEDICINI

Teaching Assistants: Michael Butler Brown and Debora Goedert

Faculty Editors: Mark E. Laidre and Matthew P. Ayres

**Abstract:** This study examined the fission-fusion dynamics of the little-known butterfly *Manataria maculata*. This nocturnal Lepidopteran roosts socially in groups of up to 50 individuals during the day, but disperses individually by night. To further understand the dynamics of *M. maculata* group formation, we observed the settling behavior of individually marked butterflies when experimentally disturbed as a group and when introduced individually to an existing group. Results revealed little consistency in *M. maculata* settling behavior and no evidence of social structure. These findings provide implications for the conservation of these butterflies and contribute to the understanding of fission fusion dynamics.

**Key words:** Fission-fusion, *Manataria maculata*, self-organization, social hierarchy

### INTRODUCTION

The organizational patterns of social organisms are key to understanding their behavior in their physical and social environments. Social groups form in many genera, varying in group size, movement patterns and stability between and within species. Groups display fission and fusion – the tendency to split and merge – daily to suit changing needs, breaking into smaller group sizes to hunt, fight, mate or forage, and returning to the larger group for benefits such as better thermoregulation or predator defense. These complicated and constantly fluctuating dynamics are relevant to the transmission of disease, the spread of genetic information, and the patterns and speed of colonization of invasive species (Couzin and Laidre 2009).

Groups are essential to the success and survival of many social insects, and for many species, leaving the group is never an option. For example, a lone termite has almost no chance of survival outside of its colony, resulting in a very stable aggregation of individuals. On the other hand, the little-studied butterfly *Manataria maculata* is a strange case of social insect. The nocturnal butterfly shares many traits with moths (Yack and Fullard 2000), but the insect is most puzzling for its unique social behavior during the dry season when it spends months at high altitude locations in sexual diapause, feeding only sporadically (Rydell et al. 2002). During the day, *M. maculata* roosts on the ground in groups of up to

50 individuals, but by night the group disperses completely as individuals seek solitary shelter in the forest canopy (Rydell et al. 2002). That these butterflies perform this nightly fission, spending 50% of their time in solitude begs the question: why re-group at all?

We evaluated group aggregation and fission-fusion dynamics in *M. maculata* with a two-part experiment. To assess the role of pre-existing groups in butterfly aggregation, we asked how an established group might influence the fusion behavior of introduced individuals. If butterflies are motivated to group due to selfish herd theory or for thermoregulatory benefits, then individuals would be expected to consistently join the largest group available. Alternatively, they may group simply because they are attracted to a common resource. To test this alternate hypothesis, we examined the butterflies' preferences between sunlit and shaded environments. In a second experiment, we considered how butterflies aggregate when there is no existing group. Do individuals exhibit personality, with some tending to be social and others to be solitary? If so, individuals would tend to return to similar positions with respect to the others in the group each time they resettle. Alternately, re-aggregation in a random pattern would suggest a lack of persistent personality types. We tested for personality by repeatedly disturbing groups of marked butterflies and recording their positions relative to others in the groups formed following these disturbances.

## METHODS

### *Aggregation Experiment*

We captured butterflies from two roosts along the trail system of the Monteverde Biological Research Station located in the high-altitude cloud forest of Costa Rica, where these butterflies rest in sexual diapause during the dry season. “Roosts” are small spaces near the ground in which groups of over 30 butterflies congregate. In this case, the roosts were a space beneath an overhang on a mudbank and a hollowed-out log. We placed a net over these spaces and disturbed the roost to flush the butterflies into the net. We kept individuals separate by group in large cylindrical butterfly traps. To evaluate how individual butterflies settled when released near an established group, we introduced 20 butterflies one at a time to a group of 14 pre-settled butterflies within a mesh cylinder 71.12cm high, 42cm in diameter, that we had not yet manipulated to simulate the return of one individual to a socially roosting group. To study light preferences, we rotated the trap after every five added individuals, ensuring that the large groups were not always in the shade or sun. We recorded whether the individual chose the light (sun) vs. dark (shade), the largest group available in the preferred light condition, or the the largest group available overall. We also measured the time it took each butterfly to settle. We repeated this process with a second group of twenty individuals, and employed chi-square tests to evaluate null hypotheses of preference for sun vs. shade, or for group vs. solitary roosting. We used an F-test to compare the settling time of butterflies which settled alone as compared to in groups.

### *Disturbance Experiment*

To evaluate whether groups and individuals within groups return to the same formations after disturbance we performed a second experiment within the same arenas. We removed ten butterflies from groups and labeled each individual with a number 1-10 on the wing using a white sharpie (4 replicates, 40 total individuals). We released all 10 individuals into an empty trap and let them settle, defining

“settling” as the point at which all butterflies stopped moving and closed their wings. Each individual’s place in the net was marked with a piece of tape listing their number and then digitally mapped by recording the X, Y and Z coordinates of each butterfly in 3-dimensional space along the internal surfaces of the arena. The group was then disturbed so that all butterflies were dislodged and timed as they resettled, and then individual locations were again remarked. We repeated this disturb/settle procedure once more, such that upon completion we had marked positions for three trials. We calculated the distance from each individual to every other individual and then all the sum of pairwise distance for each individual. This provided a metric of how aggregated or isolated individuals were positioned – larger numbers indicating an individual that settled relatively far from most others, and smaller numbers indicating an individual tending to be close to others. To analyze whether individuals return to the similar positions in the group after disturbance, we estimated the repeatability of the sum of pairwise distances across the three disturbances using the interclass correlation coefficient (ICC) of a one-way ANOVA. ICC was calculated using the package “irr” (Gamer et al. 2012) in R v3.4.1 (R Development Core Team, 2013). We also evaluated patterns in the inter-individual distances with a general linear model that included group (n=4) settling event (n=3), group x settling event, and individual within group (n=10, random effect).

## RESULTS

### *Aggregation Experiment*

Butterflies trended towards settling in a group compared to settling alone. (Fig. 1; chi-square = 3.10,  $P = 0.078$ ,  $DF = 1$ ). However, of the butterflies that had more than two groups available on their preferred side, there was no significant preference of butterflies to join the largest available group (Fig. 2; chi-square = 0.82  $P > 0.25$ ,  $DF = 1$ ), nor was there a difference in settlement time for butterflies that settled in a group vs. alone (Fig. 3;  $F_{1,36} = 0.02$ ,  $P = 0.89$ ). There was a strong tendency for butterflies to settle in sunlight vs. shade conditions (Fig. 4; chi-square = 24.64,  $P < 0.0001$ ,  $DF = 1$ ) (n=39).

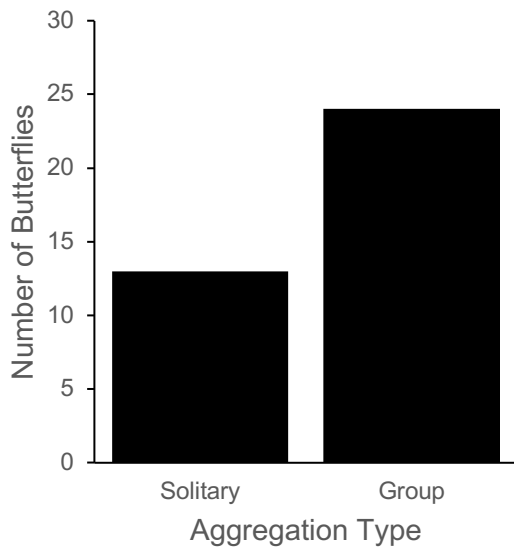


Figure 1. More butterflies settled in groups than alone.

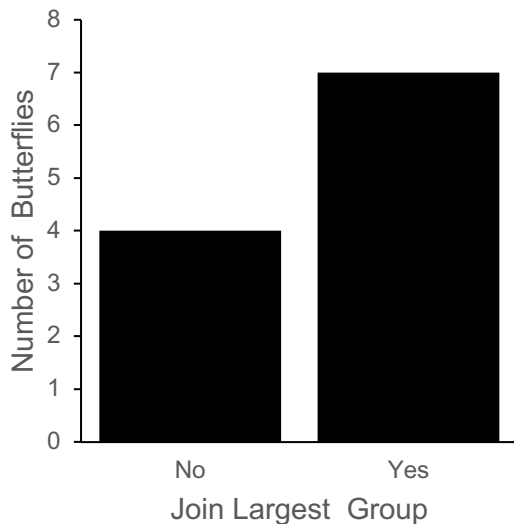


Figure 2. There was no statistical tendency for butterflies to join the larger of two groups.

#### *Disturbance Experiment*

Butterflies did not show individual personalities in grouping as evaluated with interclass correlation coefficients ( $ICC=0.002$ ,  $F_{39,80}=1.01$ ,  $P=0.48$ ) ( $n=40$ ). Neither was there evidence of visible personality from a graphical analysis (Fig. 5), or from the general linear averaged distance from each individual to others in their group (95% CI = -0.06 - 0.13). The general linear model of group aggregation patterns

suggested statistical differences among groups ( $F_{3,36} = 8.32$ ,  $P < 0.001$ ) but also an equally

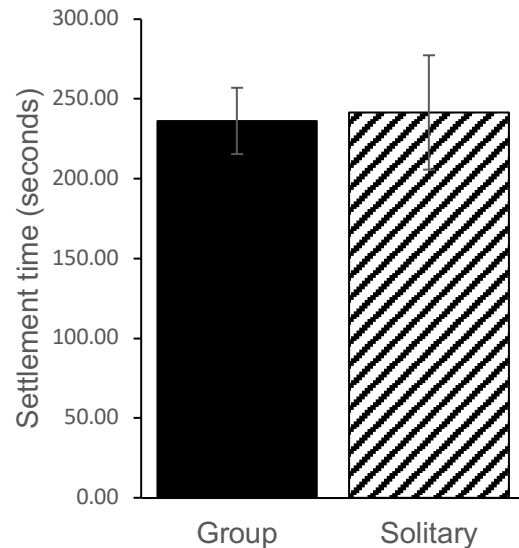


Figure 3. There was no difference in mean settlement time  $\pm$  standard error for butterflies who joined a group or settled alone ( $n=39$ ).

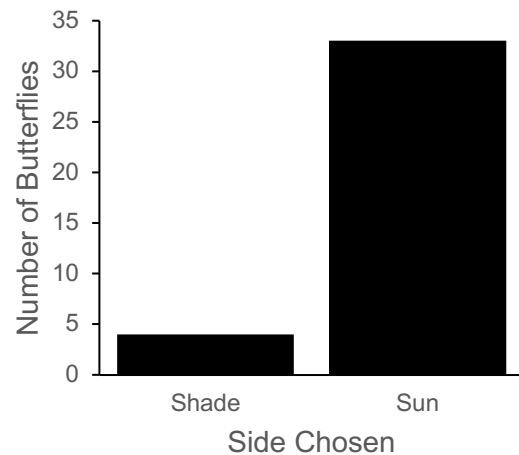


Figure 4. Butterflies showed a strong preference for settling in the sun versus shade.

strong group by settling event interaction ( $F_{6,72}=6.43$ ,  $P<0.001$ ). Graphed analysis (Fig. 6) did not indicate any meaningful main effects of either group or settling event.

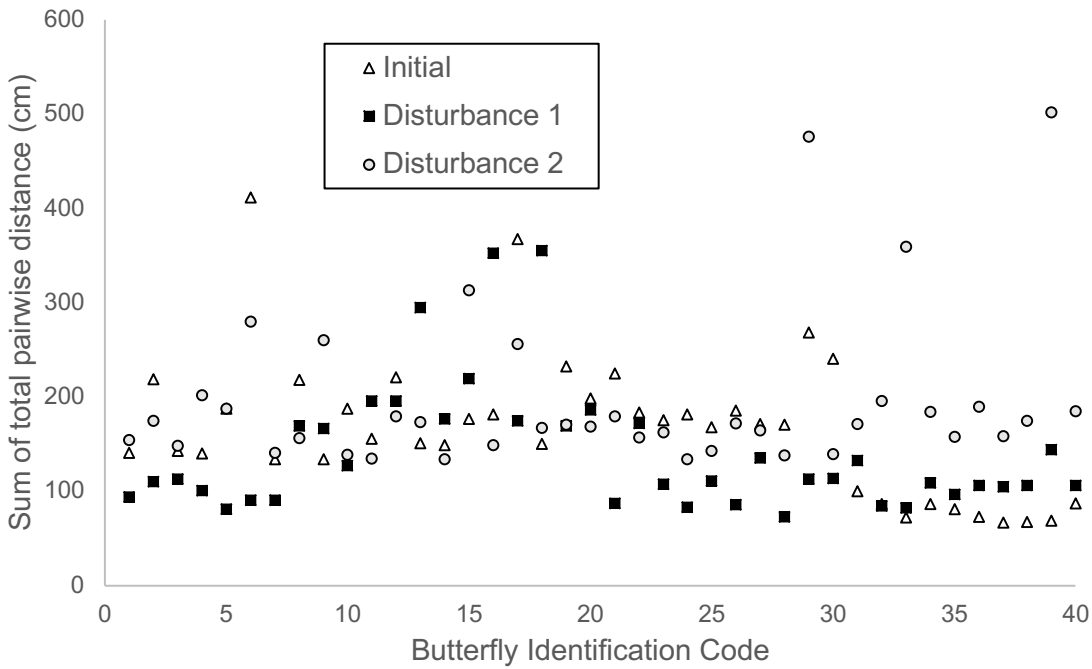


Figure 5. The sum of pairwise distances between every butterfly and the nine individuals in its respective group. There was no evidence for butterflies that preferred to roost close to versus farther away from others.

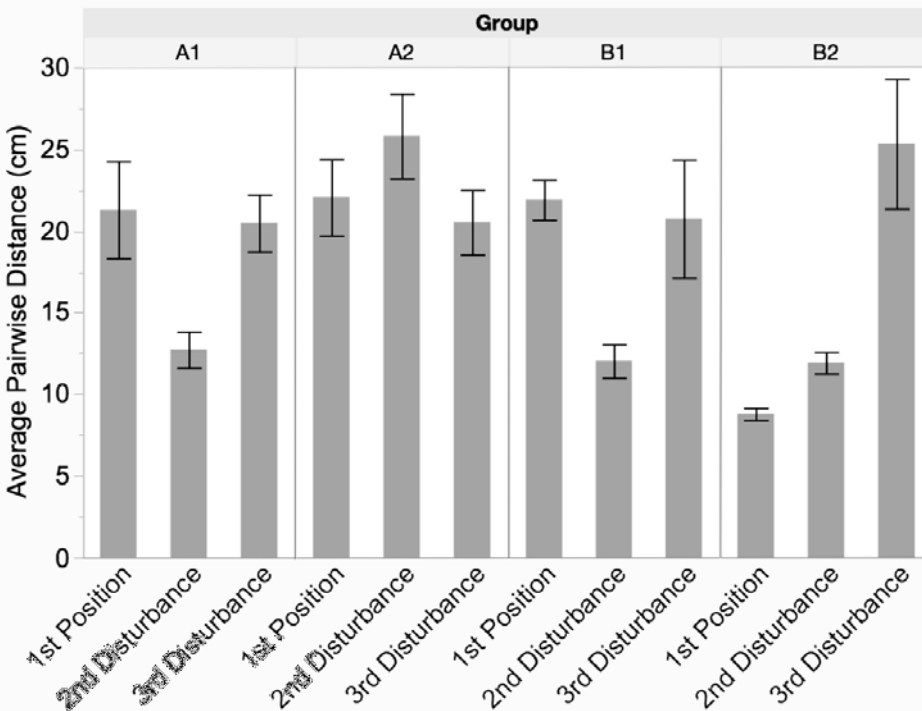


Figure 6. The average pairwise distances of four groups over three trials. There was no variation among groups or trials.

## DISCUSSION

Individual butterflies introduced to an area containing established groups tended to join a group rather than roosting alone (Fig. 1). However, they did not discriminate based on group size; when faced with a choice of two groups, individuals just as often chose the small group as the large (Fig. 2). The groups formed in our experiment (minimum of two individuals) were small compared to the naturally-formed clusters of up to 50 individuals which we observed in the field. These large groups often reform in the same roost locations across many days, even after individually dispersing to the treetops at night (Rydell et al. 2002). This reliable return suggested a cohesive group structure, but groups did not reform in predictable configurations (Fig. 6) and individuals did not consistently return to a particular position within these groups (Fig. 5). This lack of structure shows that the fission-fusion dynamics of these groups are fluid.

Apparently, there is an inherent tendency for aggregation in *M. maculata* but the size and individual make-up of these groups seems unstructured. If group-size is of no importance, it is difficult to explain the large aggregations formed during the day. A possible explanation of this contrast is that natural group fusion is a byproduct of aggregating at optimal roosting locations. Perhaps the lack of roosting locations in our experiment eliminated the cause of large group formation. However, while this may explain why butterflies did not create the largest groups possible, it does not explain why individuals clustered in many small groups instead of dispersing evenly throughout the trap. If group formation is driven solely by the common need for a certain resource, then formation of groups should cease in the absence of this resource. Butterflies may accrue advantages from inclusion in a group in addition to the advantage of a certain environmental resource. Thermoregulation and predator defense remain plausible explanations of the preference for group formation. To determine whether being in a group offers thermal rewards, temperatures of butterflies within a group could be compared to those without.

Sunlight was a stronger attractor than group size (Fig. 4). Given the choice between a large group in the dark and a small group in the light, individuals consistently chose the group in sunlight. This could be due to the artificiality of the enclosures, causing butterflies to focus on escape over roosting. However, when accounting for light conditions, butterflies still demonstrated a preference for groups over roosting alone (Fig. 1) and showed no preference for group size (Fig. 2).

Our conclusion that *M. maculata* groups lack a steady social structure is supported by our observation that an introduced individual rarely upset those in the established group. If these butterflies had hierarchies in which individuals had to compete for certain ideal positions, a new arrival's attempt to join this group would most likely disturb others. The equal settlement time of individuals that joined a group and those that chose to roost alone reveals that settling in a group likely does not consist of competition between individuals. However, we also consistently observed wing vibration upon the addition of an individual in both the introduced butterfly and those already part of the group. Wing vibration has been linked to warmth generation before flight (Rydell et al. 2002) but observed vibration in this experiment was not followed by flight and often occurred right as a new individual joined the group. We suspect that this vibration is used for communication. Communication of *M. maculata* has not been studied and examining how they utilize vibration can improve our understanding of their social interaction.

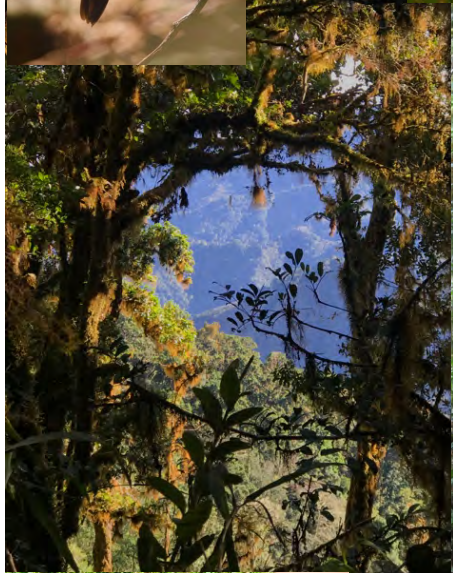
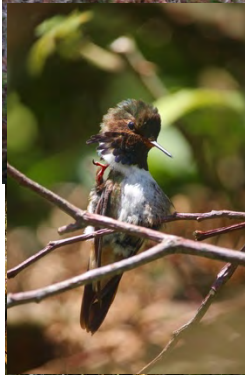
Because these social butterfly groups are not cohesive units with social structure, it is possible that individuals transfer between groups throughout their lives. However, very little is known about the migration pattern and behavior of *M. maculata* during the wet season. They roost at higher elevations including Monteverde during the dry season and travel to lowlands near the Pacific to mate (Rydell et al. 2002). Tracking movement during this period would provide information about butterfly social relationships and grouping patterns during migration.

Social dynamics between multiple roosts has not been studied, and it is unknown whether butterflies roost exclusively in one location with one group. While the lack of social hierarchy in our study makes strict separation between groups seem improbable, butterflies seem to return to the same roosts day after day. Understanding the social dynamics of these butterflies has strong implications for conservation efforts, for it is a very different project to conserve creatures strongly tied to their social groups versus those able to survive alone. Gaining a better understanding of how these loose groups influence the behavior of *Manataria maculata* can enhance the current knowledge of fission-fusion dynamics and how this system affects the organisms that display it.

#### LITERATURE CITED

- Couzin, I. D. and Laidre, M. E. 2009. Fission-fusion populations. *Current Biology* 19:15, R633-5.
- Gamer, M., Lemon, J. and Singh, I.F.P. 2012. Irr: Various coefficients of interrater reliability and agreement. <https://cran.r-project.org/web/packages/irr/irr.pdf>
- Sundaresan, S.R., Fischhov, I.R., Dushov, J. and Rubenstein, D. I. 2006. Network metrics reveal differences in social organization between two fission–fusion species, Grevy’s zebra and onager. *Behavioral Ecology* 15:140–149
- R Development Core. 2013. R: A Language and Environment for statistic computing. Vienna Austria: R Foundation for Statistical Computing.
- Rydell, J., Kaerma, S., Hedelin, H., Skals, N. 2003. Evasive response to ultrasound by the crepuscular butterfly *Manataria maculata*. *Naturwissenschaften* 90:80–83
- Yack J. E. and Fullard, J. H. 2000. Ultrasonic hearing in nocturnal butterflies. *Nature* 403: 265-6.







## PROXIMITY TO FOREST EDGE ENHANCES FLOWER VISITATION AND BLACKBERRY PRODUCTIVITY IN SMALL-SCALE AGRICULTURE

ALEXANDER W. COTNOIR, ANGELA E. ORTLIEB, CLAYTON E. JACQUES, AND MARY M. PEDICINI

Teaching Assistants: Michael Butler Brown and Debora Goedert

Faculty Editor: Matthew P. Ayres

*Abstract:* Diversification of habitats in areas surrounding agricultural fields may promote and stabilize pollination services and enhance crop productivity. We investigated the effects of proximity to forest edge on berry reproduction and the diversity of potential pollinators and natural pest-consumers in an organic blackberry field in montane Costa Rica. Alpha diversity of potential insect pollinators, abundance of birds, and blackberry pollination success and yield were positively related to proximity to forest edge. Our findings suggest that conservation of trees and forests can provide benefits to small-scale farmers, while simultaneously promoting the maintenance of biodiversity and habitat heterogeneity through the implementation of vegetative corridors.

*Key words:* Biodiversity, blackberry productivity, ecosystem services, habitat heterogeneity, optimal foraging theory, pollination, *Rubus*

### INTRODUCTION

Ecosystems provide crucial services to humans within a matrix of human-modified and natural environments. These services include water management, waste purification, and aesthetic values, among others (Ricketts 2004). In particular, the partnership between flowering crop plants and pollinators contributes to human agricultural productivity, as pollination success is associated with increased crop yields. The management and modification of this service to benefit both human and natural systems remain an active area of research within ecology and environmental studies.

Habitat diversification around crops could support greater pollinator diversity and enhance crop productivity through multiple mechanisms. The insurance hypothesis suggests that redundancy in pollinator activity across a diversity of species can increase system resilience in the face of environmental change (Winfrey and Kremen 2009). The functional complementarity hypothesis proposes that pollinators occupy non-redundant, specialized niches to support plant

productivity across temporal (e.g., seasonal or diurnal) and architectural (e.g., plant interior/exterior or low/high flowers) scales (Bluthgen and Klein 2011). Both hypotheses predict benefits of biodiversity to the ecosystem service of pollination.

Forest edges introduce habitat complexity into the otherwise homogeneous landscapes of monoculture agriculture and can support pollinator communities that benefit the agriculture. Homogeneous landscapes lack the variety of niches required to sustain a diverse population of organisms (Skórka et al. 2009). Forest corridors can provide refugia from predators while still permitting easy forays into the surrounding landscape (Hawes et al. 2008). Optimal foraging theory suggests that animals will maximize energy gain relative to foraging expenditures in pursuit of meals, implying that heterogeneous edge habitat may enhance ecological activity under certain contexts. Corridors can also connect regions of homogeneity, facilitating travel across a larger habitat and providing niches along edges that uniform habitats lack. These niches further support diverse populations of pollinators.

Blackberries (*Rubus* spp.) are an important commercial food crop in Costa Rica. They are particularly attractive to small-scale growers for the low cost of developing and maintaining orchards, the low risk of insect and disease damage, and their high nutritional value (Hussein et al. 2016). The plants are mainly pollinated by insects, although they are often visited by hummingbirds and rely upon other birds for seed dispersal (Zuchowski 2005). We conducted studies in an organic blackberry patch maintained by small-scale growers in the montane ecosystem surrounding Cuerici Biological Station, Costa Rica. This region is characterized by thick stands of oak and bamboo, which support a diversity of wildlife. Many of these montane oak forests were historically harvested for timber or cleared by cattle ranchers, and open fields still cover the lower slopes. This region receives high annual rainfall and experiences relatively cool temperatures, making it one of the few regions in Costa Rica conducive to outdoor blackberry cultivation. We studied blackberries as a model system for exploring the effects of edge habitat on flower visitation and crop productivity.

We investigated how proximity to forest edge influences the diversity of potential pollinators, the abundance of biological control agents for plant pests, and the fruiting of blackberries. We evaluated the hypothesis that proximity to forest edge promotes diversity of local insect and bird populations and results in enhanced blackberry fruiting productivity.

## METHODS

We conducted our research at Cuerici Biological Station in the Talamanca Mountain Range of Costa Rica in an organic blackberry field, bordered on northern and southern sides by secondary oak forest and bisected by a cattle trail (Fig. 1).

### *Insect community composition*

To determine whether communities of potential blackberry pollinators differed with proximity to forest edge, we deployed blue and yellow bee bowls (13 cm<sup>2</sup> square) filled with soapy water in an open area at the bases of six similarly sized blackberry bushes. We selected three bushes within 15 meters of the eastern forest edge and three at least 80 meters away from the nearest forest. We set the bee bowls at bushes that displayed mature flowers so as to attract as many potential pollinators as possible. We deployed all bee bowls on three consecutive mornings (day 1 = 8:00 am to 9:00 am, day 2 = 9:00am to 11:00 am, day 3 = 8:30 am to 10:30 am, total hours = 5) to capture potential pollinators across variations in temperature and weather. We counted and categorized the insects into morphotypes.

### *Direct observations of insects and birds*

We also directly observed animal activity and insect visitation at two locations near each forest edge and away from the edge (Fig. 1). For this, we observed similarly-sized blackberry bushes with open flowers and berries for 15 minutes on each of three mornings. We counted and identified foraging birds and insects (same morphotypes caught by bee bowls) within 5 m of the focal blackberry bush.

### *Berry and flower counts*

To determine whether pollination success and berry productivity differed with proximity to forest edge, we selected and studied 15 equally-sized bushes near the forest edge (within 15 meters) and far from the edge (at least 80 meters away). We counted the total number of berries and flowers on half of each bush, with the number of berries relative to the flowers taken as an index of the probability that a flower becomes a berry. We counted as “flower” any buds or open flowers, and as

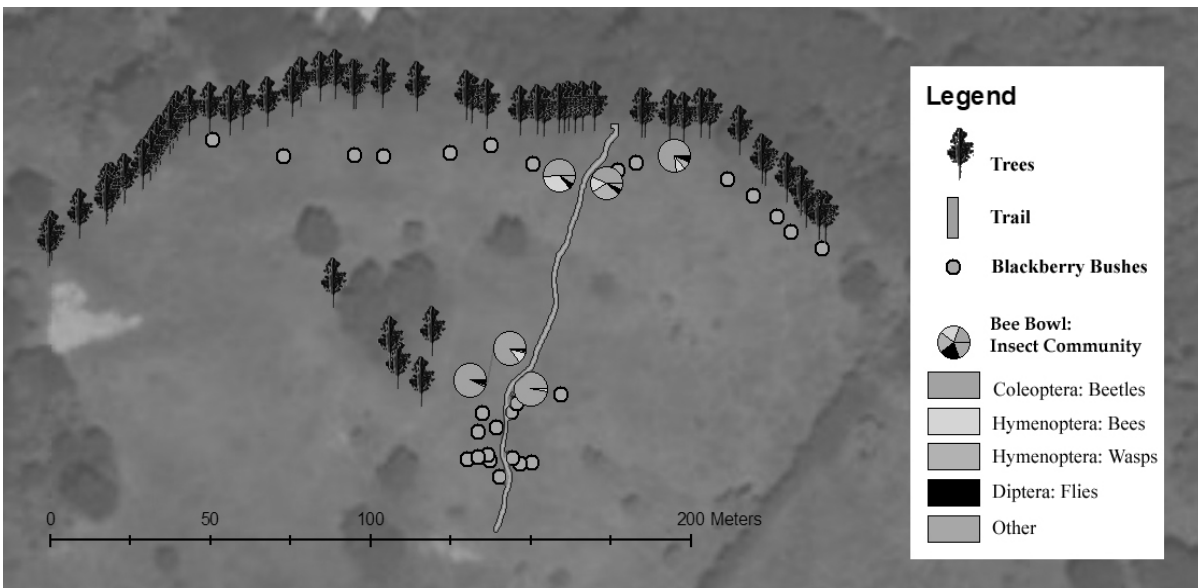


Figure 1. Map of blackberry field displaying insect diversity data collected from bee bowls at 3 bushes near forest edge and 3 bushes far from the forest edge. Bushes near forest edge had higher diversity of potential pollinating insects than bushes far from the forest edge.

“berry” all structures with fertilized drupelets regardless of age. We also examined in detail five randomly selected berries on each plant and recorded the number of drupelets that were forming. The number of drupelets per fruit was taken as an index of the proportion of ovules that had been fertilized.

#### *Wind measurements*

To compare abiotic conditions near and far from the forest edge, we recorded wind speed at each of the 30 study bushes using a Kestrel anemometer. The morning of January 26 during 0800 to 0900, we measured wind speed for 15 seconds at each bush while pointing the device into the predominant wind direction and recorded the highest wind speed during the 15 second period.

#### *Statistical Analyses*

We calculated the berry to flower ratio by dividing the number of berries found on each plant by the number of flowers. To

improve normality for statistical analyses, we first performed a natural-log transformation on the berry to flower ratios. We calculated alpha diversity as the total number of species captured across all five sampling bouts at each bee bowl. We performed t-tests to compare average berry to flower ratio; alpha diversity; proportions of wasps, bees, and beetles; and wind speed between blackberry bushes close to the forest edge and far from the forest. We performed a nested ANOVA to compare drupelet counts close to the forest edge and far from the edge: two areas (near vs. far) x 15 plants nested within area (random effect) x 5 berries per plant. All data analyses were performed in JMP Pro 13.0.

## RESULTS

### *Insect community compositions*

We captured 310 insects in bee bowls which we categorized into five taxonomic groups (Hymenoptera: Bee, Hymenoptera: Wasp, Diptera, Coleoptera, and other) and 17 unique morphotypes (Table 1). Bees and

Table 1. Broad and narrow morphotype categories of insects collected from bee bowls.

Broad Morphotype	Narrow Morphotype
Hymenoptera: Bees	Black bee
Hymenoptera: Bees	Small bee with yellow stripes
Coleoptera	Orange and black beetle
Diptera	Fly with black side spots
Diptera	House fly
Diptera	Black fly
Diptera	Tiny fly with yellow abdomen
Diptera	Red-eyed fly with pointy wings
Diptera	Tiny fly with white abdomen
Diptera	Large fly with long abdomen
Hymenoptera: Wasp	Small black wasp with curled antennae
Hymenoptera: Wasp	Tiny wasp with clear abdomen
Hymenoptera: Wasp	Black wasp with slim abdomen
Hymenoptera: Wasp	Large black wasp
Other	Brown legged leafhopper
Other	Brown spider
Other	Unicorn bug

wasps both tended to make up a higher proportion of potential pollinators near the forest edge than far from the forest edge, although neither effect was significant: for bees, mean  $\pm$  SE =  $19.8 \pm 7.8$  vs.  $4.5 \pm 3.4$  %, respectively ( $t = 1.79$ ,  $P = 0.18$ ,  $df = 2.7$ ); for wasps, mean  $\pm$  SE =  $12.2 \pm 8.9$  vs.  $1.7 \pm 0.9$  %, respectively ( $t = 1.17$ ,  $P = 0.36$ ,  $df = 2$ ).

On average, 91 % (SE =  $\pm 3.8$ ) of potential pollinators were orange and black beetles far from the forest edge, compared to only 57% (SE =  $\pm 10.2$ ) near to the forest edge ( $t = 3.14$ ,  $P = 0.06$ ,  $df = 2.5$ ). Flies comprised  $5.0 \pm 0.37$  % of potential pollinator communities near to the forest edge, compared to  $2.2 \pm 1.3$  % far from the forest edge ( $t = 2.08$ ,  $P = 0.15$ ,  $df = 4$ ). Insects that did not fall into the above categories comprised  $5.8 \pm 2.3$  % of potential pollinators near to the forest edge, compared to  $0 \pm 0$  % far from the forest edge ( $t = 2.52$ ,  $P = 0.06$ ,  $df = 4$ ).

Average alpha diversity of potential pollinators was significantly greater at blackberry bushes near the forest edge compared to far: mean  $\pm$  SD =  $7.7 \pm 1.1$  vs.  $3.3 \pm 1.1$  morphospecies ( $t = 4.6$ ,  $P < 0.01$ ,  $df = 4$ ); (Fig. 3).

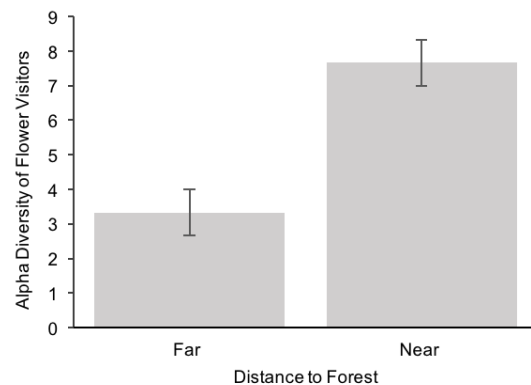


Figure 2. Alpha diversity of potential pollinators as number of unique insect morphotypes found per sample site (mean  $\pm$  SE) by distance from forest edge (far or near). There was greater diversity of potential pollinators around blackberry plants near to the forest edge compared to plants far from the forest edge. N = 3 bee bowls per distance class.

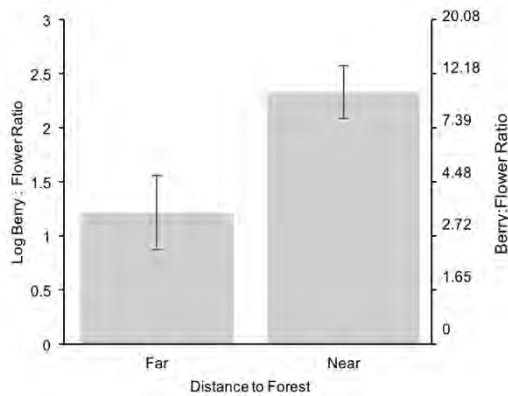


Figure 3. Log-transformed ratio of berries to flowers for individual blackberry plants (mean  $\pm$  SE) by distance from the forest edge (far or near). Secondary vertical axis on the left indicates berry:flower ratio in the original scale. N = 15 blackberry plants per distance class.

#### Direct observations of insects and birds

Near the forest edge we observed 24 hummingbirds and 31 other birds over 6.9 observation hours, while far from the forest edge we observed only 4 hummingbirds and 7 others over 5.8 observation hours. Species of hummingbirds included the White-throated Mountain Gem (*Lampornis castaneiventris*) and Magnificent Hummingbird (*Eugenes fulgens*). The species of foraging birds were mainly Wilson's Warblers (*Cardellina pusilla*), Sooty Robins (*Turdus nigrescens*), Yellowish Flycatchers (*Empidonax flavescens*), Tufted Flycatchers (*Mitrephanes phaeocercus*), and Black-capped Flycatchers (*Empidonax atriceps*).

#### Berry and flower counts

There were similar numbers of flowers on blackberry bushes near vs. far from the forest edge: mean  $\pm$  SE =  $18.9 \pm 4.3$  vs.  $22.3 \pm 3.7$  flower/ half bush, respectively. However, there were more berries on the same blackberry bushes near the forest edge vs. far from the edge:  $151 \pm 27$  vs.  $66 \pm 12$  berries/half bush, respectively ( $t = 2.88$ ,  $P = 0.01$ ,  $df = 19$ ). Thus, the ratio of berries to

flowers (Fig. 3) was significantly higher on blackberry bushes near the forest edge than bushes far from the forest edge ( $t = 2.66$ ,  $P = 0.01$ ,  $df = 25$ ). Blackberry bushes also tended to have more drupelets per berry on bushes near the forest edge than on bushes far from the forest edge (Fig. 4;  $F_{1,28} = 3.40$ ,  $P = 0.076$ ). About 20% of the random variance was among bushes within a distance class, and the remaining 80% was within bushes.

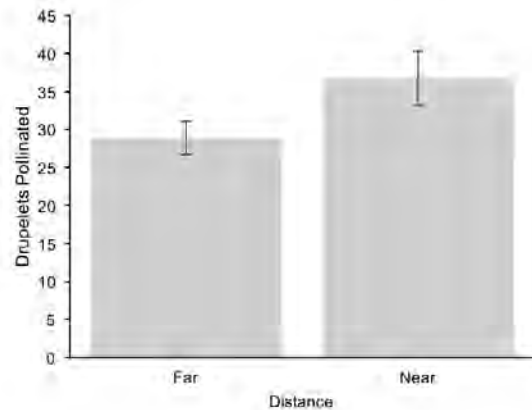


Figure 4. Number of drupelets (mean  $\pm$  SE) per blackberry far and near to the forest edge. N=15 blackberry plants per distance class, x 5 berries per plot.

#### Wind measurements

Wind speeds were significantly higher far from the forest edge (mean  $\pm$  SE =  $3.18 \pm 0.15$  m/s) than near the forest edge (mean  $\pm$  SE =  $1.47 \pm 0.29$  m/s) ( $t = 5.30$ ,  $P < 0.01$ ,  $df = 21$ ).

#### DISCUSSION

Blackberry bushes near the forest edge appeared to have greater pollination success than bushes far from the forest edge (Figs. 3-4). There was also higher diversity of insects near the forest edge versus far from the edge (Fig. 2). Together, these results are consistent with the hypothesis that forest edges increase biodiversity by providing a wide range of niches and habitats for various foragers and pollinators, and therefore

enhance berry yield in bushes close to the forest edge.

Overall, we observed more hummingbirds and insectivorous birds near the forest edge than far from the forest edge, suggesting that blackberry bushes near the forest edge may receive more visits from potential pollinators and consumers of pests than bushes farther away. The abundance and diversity of foraging birds near the forest edge may correspond to the broad range of niches and wind protection offered by the forest edge, following concepts of habitat heterogeneity and optimal foraging theory. At the far site, we noted more hummingbirds and foraging birds in our plot above the cattle trail where there were four large trees versus below the trail, farther away from any trees (Fig. 1). This suggests that even a few trees can increase local bird abundance.

Far from the forest edge, the insect community was dominated by what appeared to be a single species of orange and black leaf beetle (*Chrysomelidae*). These beetles were also found near the forest edge, but they were mixed with various species of bees, wasps, and flies. The high abundance of leaf beetles far from the forest could have been partly due to the lack of insectivorous birds foraging far from the edge. We observed flycatchers and sooty robins consuming beetles and caterpillars from blackberry leaves and flowers near the forest edge, but not far from the edge. The relationship between the leaf beetles and blackberry plants is ambiguous; one owner of the field viewed them as detrimental herbivores, while the other owner regarded them as beneficial by virtue of providing pollination services (Solano C., *pers. comm.*). There would be value in further studies of how these insects affect blackberry production and what are the factors that influence their abundance.

Preserving a diversity of pollinators is especially important given that unpredictable changes may create environmental conditions favoring an alternative species assemblage. Aggressive forms of disease and climate change pose new threats to pollinator populations. With low pollinator diversity, the loss of a single pollinator species could be devastating to pollination success and resulting crop yield. Within our study system, blackberry bushes near the forest edge may be more resilient in recovering from the loss of a single pollinator population than bushes far from the forest edge. Planting a small strip of forest habitat for perching and foraging far from the forest edge could help promote greater biodiversity and may help “insure” this region of the blackberry field against threats of pollinator collapse. There would be value in better understanding of pollination ecology and food web structure in this system.

Overall, our findings suggest that the maintenance of trees and forest fragments has value for nearby blackberry production systems. Our results are of particular interest to small-scale organic farmers and add to growing evidence that land management, such as strategic planting of forest corridors among berry monocultures, can enhance pollination success and protect crops from damaging pests. In this sense, blackberry production systems and forest conservation share common goals. It would be helpful for both forest conservation and food production to identify more such cases of complementary interests.

#### ACKNOWLEDGEMENTS

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

- Blüthgen N and Klein AM. 2011. Functional complementarity and specialisation: The role of biodiversity in plant-pollinator interactions. *Basic Appl Ecol* 12: 282–91.
- Charnov, E. L. 1976. Optimal foraging, the marginal value theorem. *Theoretical population biology* 9: 129–135.
- Hussain I, Roberto SR, Fonseca ICB, *et al.* 2016. Phenology of “Tupy” and “Xavante” blackberries grown in a subtropical area. *Sci Hortic (Amsterdam)* 201: 78–83.
- Ricketts, T. H. 2004. Tropical fragments enhance pollinator activity in nearby coffee crops. *Conservation Biology* 18-5: 1262-1271
- Skórka P, Lenda M, Martyka R, and Tworek S. 2009. The use of metapopulation and optimal foraging theories to predict movement and foraging decisions of mobile animals in heterogeneous landscapes. *Landsc Ecol* 24: 599–609.
- Winfree R and Kremen C. 2009. Are ecosystem services stabilized by differences among species? A test using crop pollination. *Proc Biol Sci* 276: 229–37.



## DOES SIZE REALLY MATTER? BODY SIZE TRADE-OFFS IN FUNNEL WEB WEAVING SPIDERS

CORINNE R. VIETORISZ AND ALEXANDRA M. STENDAHL

Teaching Assistants: Michael Butler Brown and Debora Goedert

Faculty Editor: Matthew P. Ayers

**Abstract:** In many organisms, having a bigger body size is considered advantageous, but large body sizes often come with detrimental tradeoffs. This study investigates the benefits and tradeoffs of having a large body size in funnel web weavers (Arachnida: Agelenidae) through the distribution of sizes, estimated survivorship curves, the relationship between web size and spider size, and the costs of web building. We measured the dimensions of 53 webs, the body size of 7 spiders and their webs, and rebuilding time for 15 webs. Larger spiders built larger webs, and the size distribution of spiders 7-13mm in length was about as expected for a population at steady state. Analysis of survivorship assuming a stable size distribution indicated that survival probabilities were relatively constant across sizes (Type III survivorship). Survivorship is equal across all size classes, instead of larger spiders having increased survivorship. After 48 hours, smaller spiders, but not larger spiders, tended to repair their webs to original size or larger, indicating that web building is more costly for large spiders. Apparently it is advantageous for Agelenidae to be bigger because they can build larger webs, but a tradeoff is that larger spiders take longer to repair their webs to original size. This tradeoff may help explain why larger spiders do not have an increased chance of survival.

**Key words:** Agelenidae, funnel web weaves, size, trade-offs, web building

### INTRODUCTION

It is well documented that an animal's body size often has direct consequences for fitness, generally through sexual selection in males and fecundity selection in females (Shine 1988). Selection for large body size to increase fecundity is known as Darwin's "Fecundity Advantage" model and applies to many animals such as crocodiles, fish, and insects (Thorbjarnarson 1996, Honek 1993, Hislop 1988). However, large body size can also come with tradeoffs, some significant enough that large body size is selected against. Examples of these costs include reduced survival in juveniles due to long development or fast growth, higher energy requirements, reduced agility, increased detectability, increased susceptibility to heat stress, and late reproduction (Gill 1995, Werner 1993, Blackenhorn 1995).

Spiders, like many invertebrates, exhibit increased fecundity with increased body size (Peterson 1950). However, costs of large body size in spiders are poorly studied and tradeoffs may exist that exert selection pressure towards smaller bodies. To evaluate the costs and benefits of body size in spiders, we examined

funnel web weavers (Arachnida: Agelenidae). These spiders build a complex, non-adhesive barrier web low to the ground in dark areas (Figure 1). The spider sits in a funnel retreat and waits for prey to land on the web. As this spider is dependent on its web for prey, the growth rate and body size of web occupants is likely related to web size, building costs, and local prey densities (Enders 1976, Tanaka 1989). These factors influence selection gradients for body size in web-building spiders and understanding tradeoffs would improve understanding of the evolution of body size in spiders.



Figure 1. Dorsal and ventral view of an adult funnel web weaving spider.

We investigated the benefits and tradeoffs of large body size in funnel web weavers, especially as it relates to web building. First, to determine if larger spiders have increasingly higher survival, we tested for signals of a type 3 survivorship curve (defined as increasing probability of surviving to the next size class as spiders advance to larger size classes). We predicted to see a skewed size distribution which would be indicative of a type 3 survivorship. Second, to assess whether large spiders build larger webs, we examined the relationship between web size and spider size. It could be that bigger spiders routinely build bigger webs, which would presumably catch more prey. Or it might be that web size is primarily controlled by environmental constraints that are largely independent of spider size (e.g., points for web attachment). Third, we asked how body size affects the ability of spiders to repair their webs after damage. It could be that larger spiders take longer to reconstruct damaged webs after damage because the webs are larger, or larger spiders might have mechanical advantages that allow them to build their webs faster than smaller spiders. We tested these hypotheses through studies of funnel web weavers in the montane forests surrounding the Cuerici Biological Station in Costa Rica.

#### METHODS

We located 53 active, occupied webs of what appeared to be a single species of Agelenid spider (Figure 1). We measured the length, width, and funnel diameter of each web (Figure 2). It proved difficult to catch the occupant spiders, but we were able to catch and measure the length (anterior of cephalothorax to spinneret) of seven spiders. Spider length was well correlated with the funnel diameter of webs, so we were able to estimate the size of spiders occupying all 53 webs that we measured based on the funnel diameter.

We used the frequency distribution of estimated spider sizes ( $n=53$ ) to test for the surplus of large spiders predicted if survival probabilities increase with body size. Spiders of 7mm or greater were consistent with a steady state size distribution (the size-structured equivalent of a stable age distribution, or SAD).

Therefore, we regressed  $\ln(N)$  versus size class and tested for the nonlinearity (acceleration) expected if survival probabilities increase with body size.

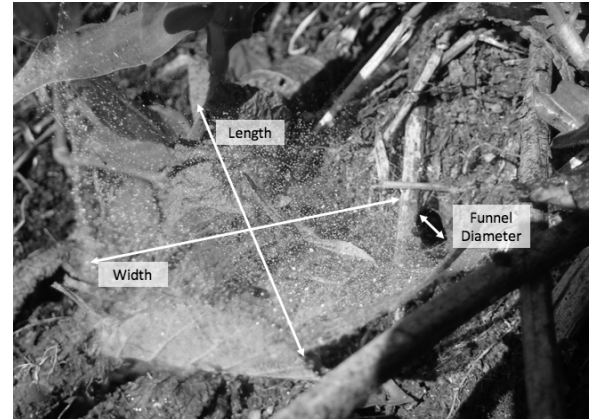


Figure 2. *Agelenidae* web with labeled width, length, and funnel diameter. Measurements were taken along the drawn lines.

We evaluated web reconstruction relative to body size by measuring and then destroying 15 active webs of variable initial sizes and then revisiting and re-measuring each web after 24 and 48 hours. We also scored completeness of webs on a scale of 1-3, where 1 = web strands present; 2 = web strong enough to hold debris or water; and 3 = web strong enough to hold debris and with a visible funnel. We regressed the size of reconstructed webs versus spider length (estimated as before from initial funnel diameter). We also regressed the ratio of repaired web area / initial web area versus spider length.

#### RESULTS

We captured 7 spiders from webs of 19 to 422  $\text{cm}^2$  in area and 6.9 to 21 mm in funnel diameter. Spiders ranged from 6 to 11.3 mm in length. Larger spiders had larger funnel diameter (slope  $\pm$  SE =  $1.42 \pm 0.19$ ,  $P < 0.001$ ,  $r^2 = 0.92$ ; Figure 3).

Based on the funnel diameters from 53 webs, the abundance of spiders per size class decreased from 7-8 mm to 12-13 mm (Figure 4). The natural log of the abundance of spiders per size class decreased linearly with increasing size class (slope  $\pm$  SE =  $-0.40 \pm 0.10$ ,  $P = 0.02$ ,  $r^2 = 0.81$ ; Figure 5).

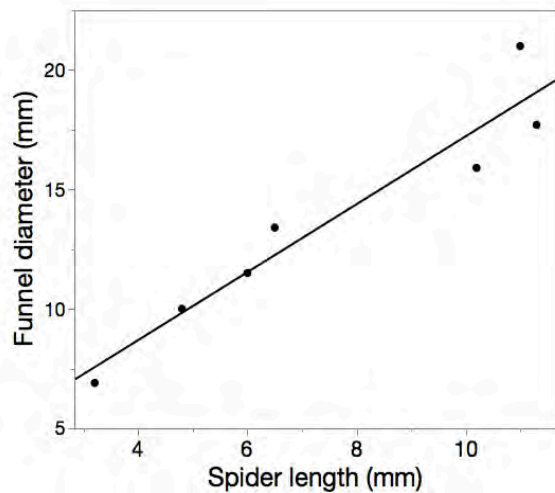


Figure 3. The funnel diameter of webs increased as a linear function of spider length ( $y = 3.01 + 1.42x$ ).

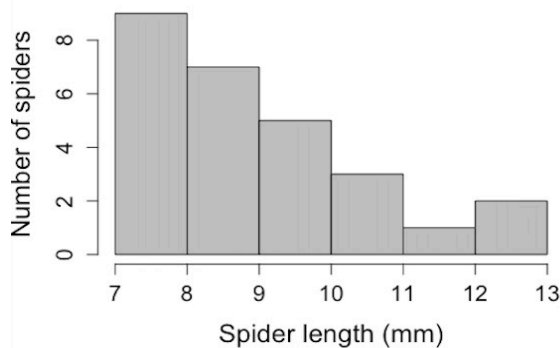


Figure 4. Frequency distribution of predicted spider length.

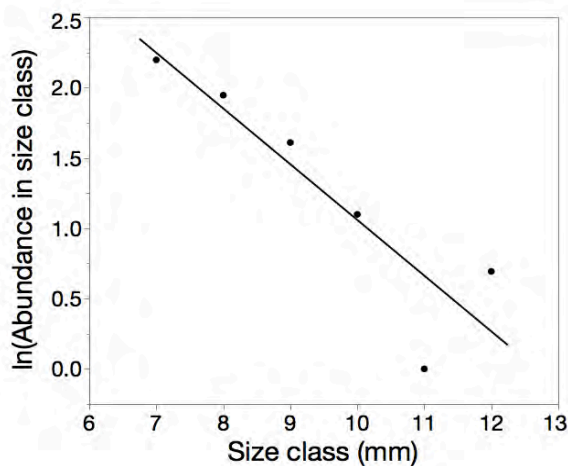


Figure 5. Natural log of the abundance of spiders in each size class. Size classes correspond to the histogram bins as in Figure 4.

The 53 webs that we measured ranged in size from 5.5 to 432 cm<sup>2</sup>. As spider size increased, web area increased (slope  $\pm$  SE =  $32.34 \pm 4.92$ ,  $P < 0.001$ ,  $r^2 = 0.46$ ; Figure 6). Variance about the line also appeared to increase as spider size increased, so large spiders had a wider range of web sizes than smaller spiders.

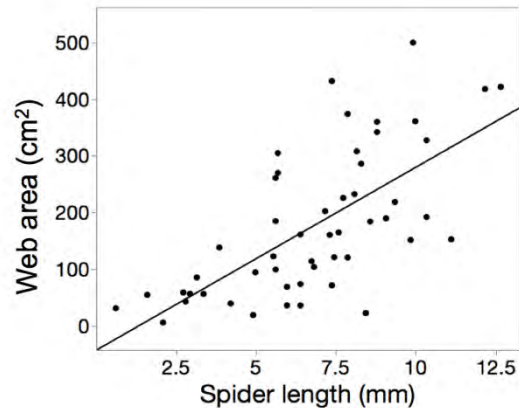


Figure 6. Relationship between web area and spider length. Bigger spiders built larger webs.

Spiders of less than about 6 mm were mostly completed with web reconstruction after 24 hours while larger spiders were still expanding and reinforcing their webs through at least 48 hours. As spider size increased, the ratio of the area of the repaired web / initial web area decreased (slope  $\pm$  SE =  $-0.99 \pm 0.30$ ,  $P = 0.006$ ,  $r^2 = 0.45$ ; Figure 7).

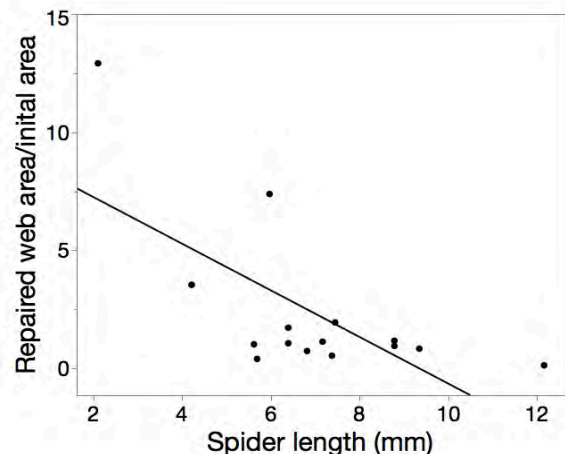


Figure 7. Ratio of the area of the repaired web/initial area of the web by spider size. The area of the repaired web / initial area indicates the proportion of the initial web that had been rebuilt 48 hours after it was initially destroyed.

## DISCUSSION

Our results indicate that larger spiders do not have higher survival probabilities than smaller spiders. That is, spider size distribution and estimated survivorship curves do not display a typical type 3 survivorship curve as hypothesized. Instead, it appeared that a spider's chance of surviving to the next size class was independent of its current size as in a type 2 survivorship function. If survival probabilities of large spiders are about the same as that of smaller spiders, as indicated by our analyses, there is neither a cost nor a benefit to larger size in terms of survival.

While being large does not offer a survival advantage, it does appear to offer an advantage in web size (figure 6). We were not able to measure capture rates as a function of web size, but it seems reasonable that larger webs catch more. If so, there is a positive relationship between spider size and web size is likely enhanced by positive feedback because bigger spiders build bigger webs, which capture more prey and permit further increases in body size. Interestingly, variance in web size increases as spider size increases, so that large spiders had a wider range of web sizes. This variation in web sizes among large individuals may be due to environmental constraints (e.g., in the size and shape of cavities available for spiders) that become increasingly relevant to a large spider.

Our results revealed at least one cost to being large: it takes a bigger spider longer to repair its web to its original size. After 48 hours, larger spiders still had repaired webs smaller than their original webs, whereas smaller spiders had already repaired their webs to original size or larger. Thus, when the web of a larger spider gets damaged, it suffers more costs than a smaller spider; if a large spider's web is not as big as it needs to be, they will catch less prey over the days it takes them to rebuild in addition to expending more energy required by building a bigger web. This rebuilding cost may be one of the factors that explain why large spiders have an equal survival rate to small spiders despite the advantage of building a bigger web and presumably catching more prey. Because rebuilding their web after damage is more costly to larger spiders, it would be advantageous for

larger spiders to build their webs in low-disturbance environments. Under this scenario, smaller spiders have higher fitness in high-disturbance environments and larger spiders have higher fitness in low-disturbance environments.

Further studies could investigate the possibility of different-sized spiders having different fitness levels in high or low disturbance environments. Additionally, our conclusions about the advantages of having a larger web could be strengthened by a more concrete link between web size and amount of prey captured. One important consideration for selection against larger size is that costs of being large occur at different points in time and space, during specific life stages and perhaps differ by sex. Understanding at what life stage the costs of being large occur would be informative. Similarly, the costs of having a large body size must be great enough so that the sporadic selection is sufficient to counterbalance perpetual and strong selection for large body size (Blanckenhorn 2000). Additional research could explore the existence of other costs to being large and at what life stage they impact the spider.

Apparently, the benefits of larger body size for funnel web weaving spiders come with tradeoffs. Although larger individuals almost certainly have increased fecundity, the size attained by a population is limited by the countervailing costs.

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## AUTHOR CONTRIBUTIONS

Both authors contributed equally.

## LITERATURE CITED

- Blanckenhorn, W. U. (2000). The evolution of body size: what keeps organisms small?. *The quarterly review of biology*, 75(4), 385-407.
- Blanckenhorn, W. U., Preziosi, R. F., & Fairbairn, D. J. (1995). Time and energy constraints and the

- evolution of sexual size dimorphism—to eat or to mate?. *Evolutionary Ecology*, 9(4), 369-381.
- Enders, F. (1976). Clutch size related to hunting manner of spider species. *Annals of the Entomological Society of America*, 69(6), 991-998.
- Gill, A. B., & Hartf, P. J. B. (1996). How feeding performance and energy intake change with a small increase in the body size of the three-spined stickleback. *Journal of fish biology*, 48(5), 878-890.
- Hislop, J. R. G. (1988). The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, *Melanogrammus aeglefinus*, in British waters. *Journal of Fish Biology*, 32(6), 923-930.
- Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, 483-492.
- Petersen, B. (1950). The relation between size of mother and number of eggs and young in some spiders and its significance for the evolution of size. *Experientia*, 6(3), 96-98.
- Shine, R. (1988). The evolution of large body size in females: a critique of Darwin's "fecundity advantage" model. *The American Naturalist*, 131(1), 124-131.
- Tanaka, K. (1989). Energetic cost of web construction and its effect on web relocation in the web-building spider *Agelena limbata*. *Oecologia*, 81(4), 459-464.
- Thorbjarnarson, J. B. (1996). Reproductive characteristics of the order Crocodylia. *Herpetologica*, 8-24.
- Werner, E. E., & Anholt, B. R. (1993). Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *The American Naturalist*, 142(2), 242-272.

## DOES POTENTIAL POLLINATION EFFICACY VARY WITH VISITOR SEX AND FLOWER TYPE?

GRACE S. CALLAHAN AND HANNAH I. HOFFMAN

Teaching Assistants: Michael Butler Brown and Debora Goedert  
Faculty Editor: Matt Ayres

*Abstract:* Although interspecific interactions can depend on intraspecific variation, flower visitors are often perceived on a binary scale as either “robbers” or “pollinators.” The former describes species that take flower resources but do not necessarily aid in flower reproduction, while the latter describes species that move pollen between conspecific flower reproductive parts. This distinction leaves little room for the possibility of intraspecific behavioral variation in different contexts. We analyzed the behavior of a common leaf beetle species that visits at least nine different flower types in the vicinity of the Cuerici Biological Station in Costa Rica. We studied beetle behavior on four flower types and compared their potential to function as pollinators depending on the sex of the beetles and the species of plant. Beetles behaved differently across the four flower types, displaying various degrees of pollen retrieval behaviors and moving with various frequencies between conspecific flowers. This behavioral variation could result in different pollination efficacies among flower types. Beetle sex did not influence behavior and thus likely does not affect pollination potentials; however, movement to conspecifics decreased with decreasing flower density, suggesting that density might influence pollination potentials.

*Keywords:* beetles, plant-insect interactions, pollination

### INTRODUCTION

Interspecific interactions range from antagonisms to mutualisms, and intraspecific variation can change where relationships lie along this spectrum (Kula et al. 2014). Nonetheless, flower visitors are often classified as “robbers” or “pollinators,” a binary outlook that discounts possible gradients within these categories. A robber takes flower resources but does not necessarily aid in flower reproduction, while a pollinator – at minimum – retrieves pollen, moves to conspecific flowers, and transfers pollen to conspecific reproductive parts (Biology of Plants; Kevan 1983). Although pollination is frequently perceived in this manner, the breadth of animal behavioral repertoires suggests that more intricacy exists in flower-animal interactions. For instance, a single species of visitor could exhibit varying degrees of pollination effectiveness.

Several factors could influence animals’ roles as pollinators. Intraspecific behavioral variation could impact interaction with flowers; for instance, males might move among flowers more frequently to find females, coincidentally increasing the potential for pollination (Cane 2001). Conversely, males within flowers might

spend more time soliciting mating opportunities and less time foraging for pollen and nectar, thus providing decreased pollination effectiveness. Another potential influence on pollination are differences among flower type and plant species. For example, plant species with higher densities of flowers within patches might be more likely to be pollinated even given the same morphology and behavior of visitors (Elliot et al. 2009).

We studied the potential for differences in pollination efficacy of an insect visitor species depending on the sex of the insect and the type of flower. A species of leaf beetle (Coleoptera: Chrysomelidae) that is common near the Cuerici Biological Station in Costa Rica provided a model system with which to investigate these possibilities; we observed adults of this beetle visiting at least nine different flower types in the same immediate area. By observing these leaf beetles across four flower types, we analyzed behavioral variation and developed an index to evaluate their potential as pollinators. We evaluated possible effects from the sex of beetles and the density of the various flower types that the beetles visit.

## METHODS

We studied beetle behavior on four locally common flower types, all in the family Asteraceae: a type of dahlia, a white daisy, a dandelion (genus *Taraxacum*), and a type of geranium (Figure 1). Observations began by randomly choosing one of the four flower types, finding a patch of that type along the trail, then walking a random number of steps from 1 to 10 and choosing the closest beetle on the selected flower type. We observed each focal beetle for five minutes and noted each occurrence of five possible behaviors: inserting their heads into the inflorescence, grazing on disks (“petals”), grooming their bodies and eating pollen from it, resting on discs of the inflorescence, and copulating. We recorded the sex of the beetle. We also recorded any movements to conspecific flowers and whether or not it touched conspecific flowers’ reproductive parts. For each beetle, we calculated the proportion of each behavior’s occurrence (e.g., if an individual grazed and cleaned during the interval, ½ of its behavior was grazing and ½ of its behavior was cleaning). We then compared the proportion of occurrences of each behavior on each flower type.

To evaluate whether leaf beetle pollination potentials differ across flower types, we created a pollination potential index based on the three criteria for pollination: pollen retrieval, pollen movement to conspecific flowers, and pollen transfer to reproductive part(s) on the conspecific flower (Equation 1). From in-field observation and by examining pollen quantities on beetles under a microscope, we determined that beetles gain the most pollen when they insert their heads into flowers, less when they graze, and none when they groom, rest on petals, or copulate. We assigned each behavior a valence based on these relative amounts of pollen retrieval: 2, 1, 0, 0, and 0, respectively. To account for pollen retrieval, we multiplied each behavior’s valence by one if it occurred in the five-minute observation interval and by zero if it did not; we then divided the sum of these values by the total number of behaviors observed. To account for pollen movement to conspecific flowers, we multiplied the pollen retrieval rating by the number of times the given

individual moved to a conspecific flower per minute. To account for pollen transfer to reproductive part(s) on the conspecific flower, we multiplied this result by zero if the beetle did not touch the reproductive parts and by one if it did, as pollination is not possible if this action does not occur. The product of this equation provided the pollination potential rating for one beetle in the observation interval, with higher values representing greater pollination potential.

$$\begin{aligned}
 & \text{Pollination potential} \\
 &= \left[ \frac{2 \times \begin{pmatrix} 1, \text{if inserted head} \\ 0, \text{if not} \end{pmatrix} + \begin{pmatrix} 1, \text{if grazed} \\ 0, \text{if not} \end{pmatrix}}{\# \text{ activities performed}} \right] \\
 &\times \left[ \frac{\# \text{ movements made to conspecific flowers}}{\text{time (min)}} \right] \\
 &\times \left[ \begin{pmatrix} 1, \text{if touched reproductive parts on conspecific} \\ 0, \text{if not} \end{pmatrix} \right]
 \end{aligned}
 \tag{1}$$

We calculated the pollination potential ratings for each beetle and used an ANOVA to determine the effect of flower type on pollination potential ratings. We conducted post-hoc contrast tests to compare pollination potential scores across each possible pair of flower types.

To evaluate the effect of beetle sex on behavior variation across flower types, we observed five males and five females on each of four flower types and recorded which of the five behaviors were exhibited over five minutes and how many movements each individual made to conspecific flowers. We found only four and one female(s) to observe on geraniums and dandelions, respectively. We calculated the proportion of each behavior individuals exhibited by dividing a demonstrated behavior (quantified as the value one if it was displayed) by the total number of behaviors displayed. For each behavior, we ran a one-way ANOVA to evaluate the effects of sex, flower type, and the interaction between these two on the proportion of each behavior. We ran a two-sample t test to compare the frequencies with which females and males moved between conspecific flowers.





Figure 1. The four flowers on which leaf beetle behavior was examined: (a) dahlias; (b) daisies; (c) dandelions; (d) geraniums.

To investigate the effect of flower density on the frequency of beetle movements between conspecific flowers, we made observations in replicate plots (0.75 x 0.75 m) along the trail. When we saw a single leaf beetle on a focal flower type, we placed the plot such that the beetle was located in the center. When we saw more than one leaf beetle on a focal flower type, we placed the plot by moving it between 0 and 20 centimeters (selected randomly) from the edge of the beetle patch. We collected data for 6-8 plots of each flower type. Within each plot, we recorded the number of the focal flower type, the number of beetles, and the number of movements between conspecific flowers of the focal type that the beetle(s) made in five minutes. We calculated the average number of beetle movements between conspecific flowers of a focal type by dividing the total number of beetle movements to conspecific flowers by the total number of beetles. We used an ANOVA to test for differences in flower densities among flower types and followed this analysis by post hoc pairwise contrasts. We subsequently ran a linear regression for all plots of the number of movements between conspecific flowers and flower density. Flower densities were log-transformed. The measurements for one plot were excluded from analysis because the number of movements that one beetle made between conspecific flowers was more than 1.5 times the interquartile range greater than the next highest value in the series. All analyses were conducted using JMP13 software.

## RESULTS

Beetles displayed pollen retrieval behaviors (i.e., inserting heads and grazing) most frequently on

daisies, followed by geraniums, dandelions, and dahlias (Figure 2). Copulation accounted for more than one-quarter of the behaviors observed on dahlias, whereas it accounted for only 5% of behavior on daisies and none on dandelions or geraniums (Figure 2).

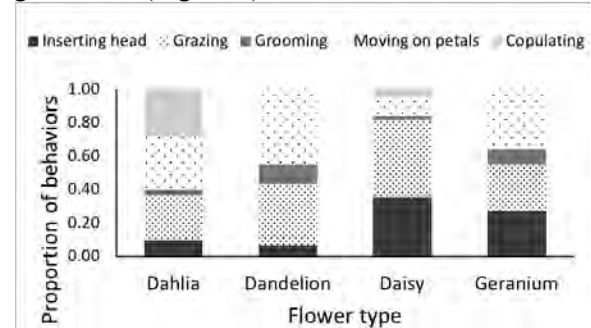


Figure 2. Proportions of leaf beetle behaviors across four flower types. (N=10 beetles for each flower type)

The average index of pollination potential of beetles varied ( $F_{3,36} = 5.87$ ,  $P < 0.05$ ); estimated potential was lower for dahlias than for daisies and geraniums and was significantly lower for dandelions than geraniums ( $P < 0.05$ ) (Figure 3).

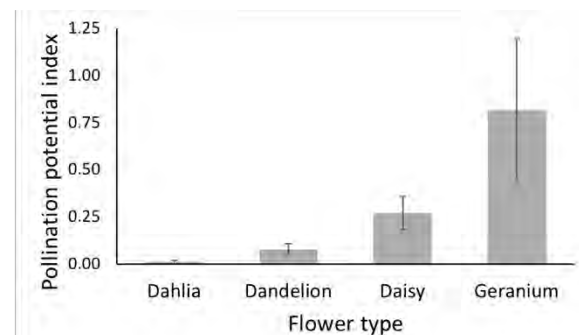


Figure 3. Leaf beetle pollination potential varied across flower types (see Equation 1). Standard error bars are displayed. (N=10 beetles for each flower type)

Sex did not have an effect on the proportion of behaviors exhibited by beetles on each flower type and there was no main effect of sex by flower type interaction for any of five behaviors ( $F_{1,28} < 2.04$ ,  $P > 0.16$ ) (Figure 4). Nor was there any difference between the frequency with which beetles of each sex moved between conspecific flowers ( $t = 0.88$ ,  $P > 0.05$ ,  $df = 34$ ).

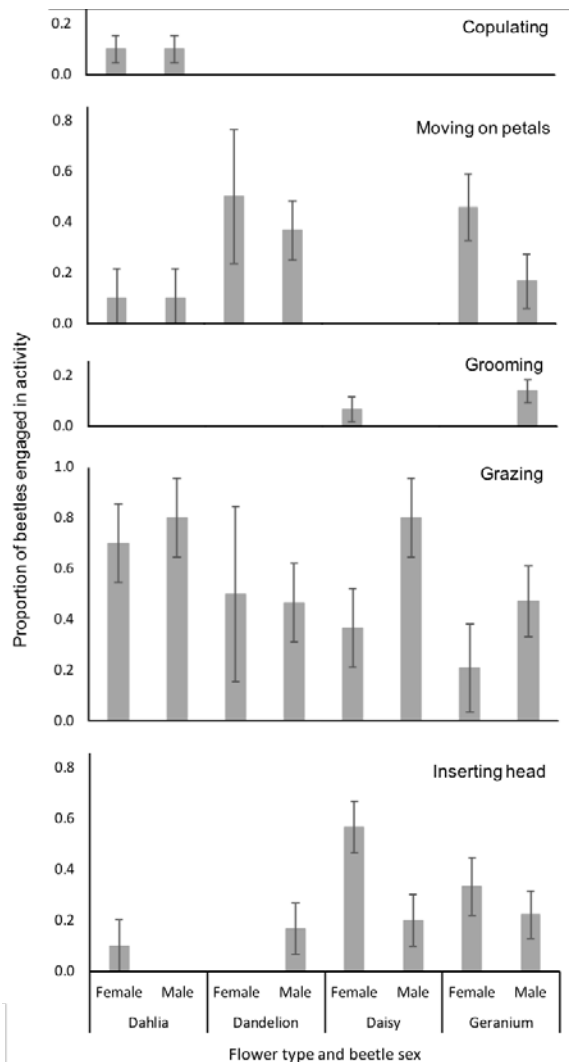


Figure 4. Proportion of female and male beetles engaging in each of five different activities on four flower types. (N=10 beetles for each flower type)

Flower types varied in density: mean  $\pm$  SE =  $5.2 \pm 0.9$  flowers/m<sup>2</sup>,  $11.7 \pm 2.5$  flowers/m<sup>2</sup>,  $18.0 \pm 2.9$  flowers/m<sup>2</sup>, and  $27.5 \pm 5.0$  flowers/m<sup>2</sup> for dahlias, dandelions, geraniums, and daisies, respectively ( $F_{3,34}=13.19$ ,  $P<0.01$ ). Dahlias were occurred at lower densities than the other three

species, and dandelions occurred at lower densities than daisies ( $P<0.05$ ). Leaf beetles moved to conspecific flowers more frequently when flower density was greater (linear regression:  $F_{1,36}=6.60$ ,  $P < 0.05$ ,  $r^2 = 0.16$ )

## DISCUSSION

A leaf beetle species varied in its behavior as a flower visitor depending on the host flower type. This finding indicated that pollination potential differed among flower types. The beetles seemed to provide less potential benefit to dahlias than other flower types because they spent less time retrieving pollen on this type and were less likely to move between dahlia flowers. On dandelions, beetles were more likely to retrieve pollen and move between conspecific flowers than on dahlias; nonetheless, pollination potentials of these flower types were comparable. Beetles retrieved pollen more frequently on daisies than geraniums but moved between geranium flowers more than between daisies; however, we cannot estimate whether there is a difference in pollination potential between these two flower types.

There was no evidence that sex of the beetles had any effects on behavior that would translate to differences in pollination efficacy (Figure 4). However, females were more difficult to find than males in the field. Perhaps individuals of different sexes forage at various times or locations. It is unknown how this unequal abundance of males and females affects flower pollination. Males will eventually have to find females to mate with, perhaps causing them to travel more frequently between conspecifics and thus transfer more pollen. Further study could yield better understanding of how sex of flower visitors influences pollination. There was a conspicuous effect of flower density on movement between conspecific flowers, suggesting that flower density might be a key factor in pollination potential.

While our pollination potential index allows for deeper understanding of how the behavior of visitors can affect pollination, adjustments could improve this methodology. Future studies could evaluate the relationship between pollination potential and pollination effectiveness to determine whether the former accurately

predicts the latter. Furthermore, the valence system of the index, which ranks pollination behaviors from neutral to increasingly positive, does not account for possible parasitic behavior. Studies could quantify the amount of pollen consumed per beetle per day, the amount of pollen transferred per beetle per day, pollen replenishment time of each flower type, and the amount of pollen that must be transported to ensure successful flower reproduction.

Through this study of leaf beetles, we have demonstrated that a single species of flower visitor can vary in the pollination services that it provides to plants. These results encourage context-dependent inquiries of other interspecies interactions that transcend the traditional molds of mutualisms, commensalisms, and parasitisms.

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#### AUTHOR CONTRIBUTIONS

Both authors contributed equally.

#### LITERATURE CITED

- Cane, J. H. 2001. Pollinating bees (Hymenoptera: Apiformes) of U.S. alfalfa compared for rates of pod and seed set. *Journal of Economic Entomology* 95: 22-27.
- Elliot, S. E. and R. E. Irwin, 2009. Effects of flowering plant density on pollinator visitation, pollen receipt, and seed production in *Delphinium barbeyi* (Ranunculaceae). *American Journal of Botany* 96: 912-919.
- Kevan, P. G. 1983. Insects as flower visitors and pollinators. *Annual Review of Entomology* 28: 407-453.
- Pollination. *Biology of Plants*. Published online at <http://www.mbgnet.net/bioplants/pollination.html>, accessed 2/3/18 [Website]
- Kula, A. A. R., D. M. Castillo, M. R. Dudash, and C. B. Fenster. 2014. Interactions between a pollinating seed predator and its host plant: the role of environmental context within a population. *Ecology and Evolution* 4(14): 2901-2912.

## SEEING RED: AN ANALYSIS OF REDNESS IN BROMELIADS

MAANAV JALAN, PAUL J. VICKERS, BALTHASAR VON HOYNINGEN HUENE

Teaching Assistants: Michael B. Brown and Debora Goedert

Faculty Editor: Matt Ayres

**Abstract:** Red coloration in organisms can be adaptive or be neutral. Plants express redness through the production of anthocyanins, pigments that can protect cells from ultraviolet radiation. We tested for adaptive expression of redness by studying an epiphytic bromeliad *Vriesea*, which produces varying levels of foliar anthocyanins in montane ecosystems of Costa Rica. Bromeliads growing in environments with higher solar radiation, i.e., in open trees and higher up in trees were redder than bromeliads growing in low radiation environments. Additionally, we found no relation between redness and developmental stability of bromeliad whorls, consistent with the hypothesis that anthocyanins provide effective protection from UV light. Our results suggest that anthocyanins can be adaptive in bromeliads in this environment, but do not imply that anthocyanins are adaptive wherever they occur.

**Key words:** anthocyanins, bromeliad, developmental stability, ultraviolet radiation, *Vriesea*

## INTRODUCTION

Many organisms express red colors for adaptive or neutral reasons. Redness in plants is typically caused by the pigment anthocyanin, the role of which remains highly contested (Landi et al. 2015). For any plant population, there could be fitness benefits from the pigment, or its expression may be due to random genetic expression without changes in fitness (Landi et al. 2015). One function of anthocyanins can be their ability to absorb ultraviolet radiation that would otherwise be harmful to the plant tissue (Stapleton 1995, Landi et al. 2015). This function may be particularly salient in environments with high solar radiation.

Leaf development, among other physiological processes, can be negatively affected by ultraviolet radiation (Caldwell et al. 1995). Irradiation results in damage to plant DNA, proteins, and membranes, which can disrupt leaf growth (Jansena et al. 1998). Plants that resist radiation are expected to display greater developmental stability in environments with high incidence of ultraviolet radiation (Freeman et al. 1993). Plant species that have experienced high radiation may display adaptations to lessen the detrimental effects (Jansena et al. 1998).

We evaluated adaptive vs. neutral explanations for variation in the leaves of a common epiphytic bromeliad, probably *Vriesea* sp. around Cuerici Biological Station, Costa

Rica. The study site was located at an elevation of 2650 m and thus was exposed to relatively high ultraviolet radiation (Blumthaler et al. 1997). If anthocyanin production is an adaptation to shield bromeliads from ultraviolet radiation, one could expect higher redness in individual plants exposed to particularly high radiation, such as on high branches of trees or in open canopy. If UV radiation damages plant tissue, one could expect reduced stability in plants that are unprotected (low redness) or in microhabitats with high radiation.

## METHODS

*Sun exposure*

We measured 34 *Vriesea* sp. along the trail above Cuerici Biological Station. All bromeliads appeared to be a single species and were distinguishable based on leaf morphology. We categorized the bromeliads we measured as growing in low ( $n = 20$ ) or high radiation ( $n = 14$ ) based on a visual inspection of the canopy around each plant. We recorded height on the trees using a Nikon Forestry 550 Hypsometer. Where vegetation interfered, we estimated height using a photograph of the tree with a meter stick for scale.

We took photographs of each bromeliad using a Canon 7D camera with a 400 mm lens and a wide-angle photo from below the bromeliad of the overhead canopy using an iPhone 6. To quantify redness of each plant, we

analyzed the photographs in Adobe Photoshop CS5. First, we selected large evenly-lit portions from three leaves in a bromeliad. Then, we averaged the color of each selection using the Blur[Average] function and recorded the brightness intensity for red, green, and blue channels (scale of 0 to 255). With these values, we calculated the redness of each selection using the formula  $r/[(r+g+b)/3]$ , which compensated for variation among image brightness (Barber et al. 2000). We applied this formula to each of the three sample leaves of a bromeliad and averaged to obtain a score for the plant's redness value. From the canopy photograph, we estimated percent canopy cover using ImageJ software. We converted each photograph into a black-and-white image that separated canopy cover from sky, made manual corrections to the black-and-white images, and then used pixel counts to estimate the percentage of each image covered by canopy. These measurements were consistent with our binary classification of low vs. high light ( $t = 5.44$ ,  $P < 0.001$ ,  $df = 22.5$ ) so further analysis employed the categories of low vs. high light. We used a two-sample t-test to compare redness scores of bromeliads in high and low radiation conditions and calculated a linear regression of redness vs. bromeliad height.

#### *Developmental stability*

To test the relation between redness and developmental stability, we analyzed 12 fallen *Vriesea* sp. that varied in redness. We photographed each with the same focal length, aperture, and light and quantified redness as before. Next, we removed the third innermost leaf through the eighth leaf in the bromeliad whorl and measured their lengths and widths at 25 percent, 50 percent, and 75 percent along the leaf length. These five leaves were chosen because they captured a developmental progression of increasing age and size that permitted quantification of developmental stability. We performed a principal components analysis of the four leaf measurements to reduce the dimensionality. The first principle components axis was interpretable as a measure of leaf size and the second principle components axis as a measure of leaf shape (Table 1). This left us with measurements of PC1 and PC2 for

each of the five leaves per plant that were of ascending size and age. We then fit two general linear models (one to predict PC1 and another to predict PC2) that included as predictors position within whorl as a continuous variable, and bromeliad as a categorical random effect. The relationship between PC1 and whorl position was slightly nonlinear, so we included a quadratic term (whorl position squared) to this model (Supplemental Figure 1). We used residuals from these models to calculate for each plant two measures of developmental stability, one reflecting stability in leaf size (from PC1) and the other stability in leaf shape (PC2). The standard deviations for each plant in the residuals of PC1 and PC2 were interpreted as indices of developmental instability in leaf size and leaf shape, respectively. The inverse of these standard deviations was therefore interpretable as developmental stability. We used linear regressions to evaluate relations between redness and these measures of developmental stability. We plotted these inverse standard deviations of leaf size and shape against the bromeliad's mean redness using linear regressions.

Table 1. Loading results of the PCA.

	<b>PC1</b>	<b>PC2</b>
<b>Length</b>	0.42	0.84
<b>Width at 75 % leaf</b>	0.52	-0.02
<b>Width at 50 % leaf</b>	0.51	-0.52
<b>Width at 25 % leaf</b>	0.54	-0.14
<b>%Variation</b>	76.9	15.7

## RESULTS

### *Sun exposure*

Bromeliads in high radiation environments were redder (by about 16 percent) than those in low radiation environments (Figure 1;  $t = 3.86$ ,  $P = 0.001$ ,  $df = 28.6$ ). Additionally, redness was positively correlated with height on tree (Figure

2; slope  $\pm$  SE =  $1.16 \pm 0.05$ ,  $P = 0.005$ ,  $r^2 = 0.23$ ).

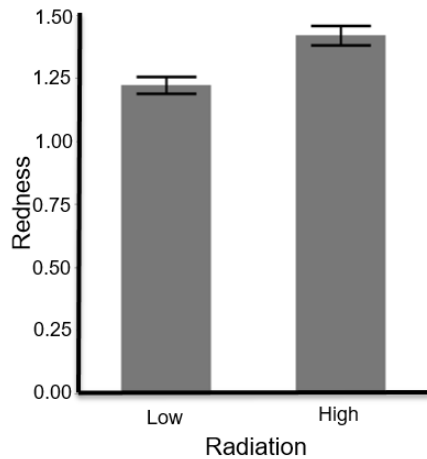


Figure 1. Bromeliads growing in high radiation were redder than those growing in environments with lower radiation. Error bars represent standard error.

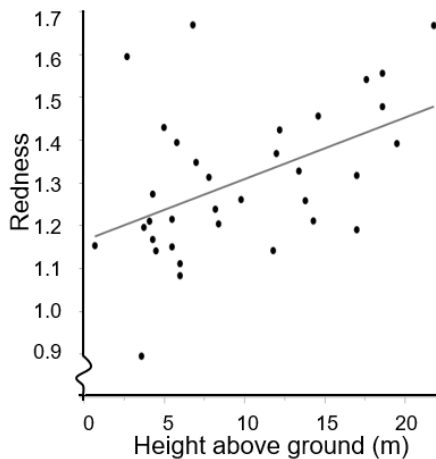


Figure 2. Bromeliad redness increased with height on tree.

#### *Developmental stability*

There was no detectable relationship between developmental stability and the redness of bromeliads (Figure 3;  $F_{1,10} = 2.14$ ,  $P = 0.17$ ; Figure 4;  $F_{1,10} = 3.40$ ,  $P = 0.10$ ).

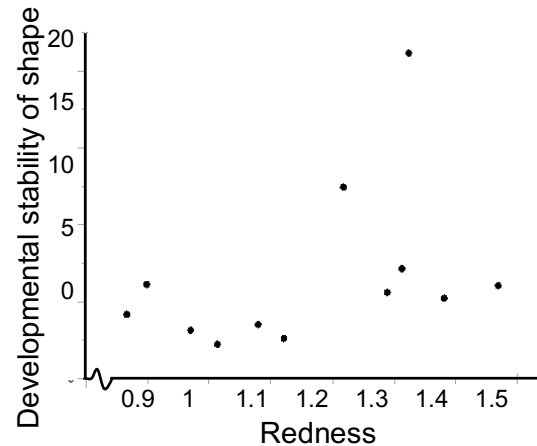


Figure 4. Developmental stability of leaf shape as a function of bromeliad redness. Developmental stability =  $1/SD$  (Residuals in Supplemental Figure 1, right).

#### DISCUSSION

Bromeliads in environments with higher radiation expressed higher anthocyanin production, supporting an adaptive role of redness as ultraviolet protection. This sorting of redness in bromeliads could be a result of phenotypic plasticity (upregulation of anthocyanins with exposure to high radiation) or differential survival in different light environments of genotypes that vary in redness. Phenotypic plasticity would imply that all bromeliads in the species carry genes for anthocyanin production but are activated only under certain radiation conditions. Alternatively, redness in each bromeliad may be genetically fixed, with redder plants having higher survival in high radiation environments such as in open canopy. Because anthocyanin production requires energy, bromeliads that are redder than they need to be could be outcompeted by greener bromeliads in low radiation environments. Future studies could test for a response in redness following transplantation of bromeliads into different light conditions. The hypothesis of adaptive phenotypic plasticity would be supported if bromeliads produce more anthocyanins and became redder after being moved from low to high radiation environments.

The developmental stability of bromeliads did not vary with redness. Given that we found redder bromeliads in high radiation



environments, this suggests that the anthocyanins in exposed bromeliads successfully compensate for increased UV radiation. Alternatively, ultraviolet radiation may not impact bromeliad development in ways that our methods could have revealed. Had our results shown a negative slope, they would have indicated that anthocyanins are not able to fully protect bromeliads from ultraviolet radiation damage. On the other hand, a positive slope may have indicated extraneous investment in anthocyanin protection. We sampled bromeliads from a recently fallen tree as we did not have the means of collecting bromeliads from erect trees, and could not account for the original conditions of the bromeliads in our analyses. Further studies could repeat this analysis taking into consideration the radiation condition of sampled bromeliads to be able to make definite inferences about radiation exposure and developmental stability in bromeliads. It would also be informative to look for evidence of developmental instability in *Vriesea sp* where they can be found in environments with even higher radiation than our study area (e.g., further up the mountains of Cerro de la Muerte to as high as *Vriesea sp* can be found).

We found evidence that anthocyanins function for ultraviolet protection in the high elevation regions of Costa Rica. This could extend to other plants in similar high radiation environments. Still, our results do not imply that anthocyanins are adaptive wherever they occur. Anthocyanin expression may range from adaptive to neutral depending on the environment.

#### ACKNOWLEDGEMENTS

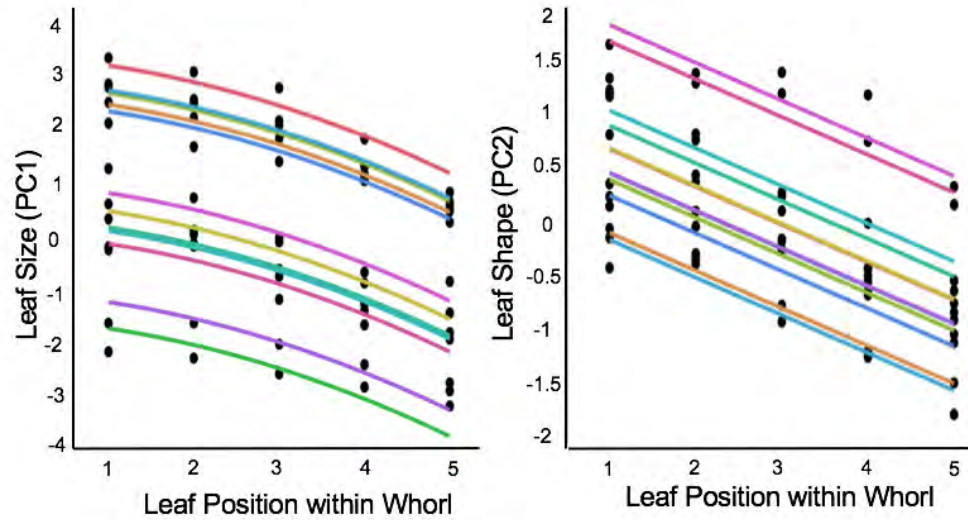
We thank Matt Ayres, Michael B. Brown and Debora Goedert for their guidance and support, as well as the Cuerici Biological Station for accommodation and research facilities.

#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

- Barber, I., Arnott, S. A., Braithwaite, V. A., Andrew, J., Mullen, W. and F. A. Huntingford. 2000. Carotenoid-based sexual coloration and body condition in nesting male sticklebacks. *Journal of Fish Biology* 57: 777-790.
- Blumthaler, M., R. Ellinger. 1997. Increase in solar UV radiation with altitude. *Journal of Photochemistry and Photobiology* 39: 130-134.
- Freeman, D.C., Graham, J.H., and Emlen J.M. 1993. Developmental stability in plants: symmetries, stress and epigenesis. *Genetica* 89: 97-119.
- Jansena, Marcel A.K., V. Gaba, and B. M. Greenberg. 1998. Higher plants and UV-B radiation: balancing damage, repair and acclimation. *Trends in plant science* 3: 131-135.
- Landi, M., Tattini, M., and K. S. Gould. 2015. Multiple functional roles of anthocyanins in plant-environment interaction. *Environmental and Experimental Botany* 119: 4-17.
- Stapleton, A.E. 1992. Ultraviolet radiation and plants: Burning questions. *Plant Cell* 4: 1353-1358.



Supplemental Figure 1. Leaf size (left) and leaf shape (right) as a function of leaf position within a whorl for 12 bromeliad plants. Each line represents one bromeliad. The standard deviation of residuals for each bromeliad was taken as a measure of developmental instability.

## MICROBIAL OXYGEN CONSUMPTION IN RAINBOW TROUT AQUACULTURE

ALEC B. COBBAN

Teaching Assistants: Michael B. Brown and Debora Goedert

Faculty Editor: Matt Ayres

*Abstract:* Microbial communities can have large impacts on ecosystem function. In the case of aquaculture, an important metric of system health is dissolved oxygen (DO). DO exits from an aquatic ecosystem by respiration of both macrofauna and microbes. The microbial impact usually depends on carbon inputs through primary productivity and sedimentation of organic particles. I evaluated the effects of sedimentation on microbial respiration in a trout farm, which consisted of several water-filled trenches connected in a series that suggested potential for different amounts of sedimentation in each different trench. I hypothesized that microbial respiration would reduce DO, and that the rate of respiration would be higher in pools with higher amounts of suspended sediment. I measured the sediment composition of the water column for each trench and measured the associated microbial respiration rates, through incubation experiments. Trenches varied in their sediment make up and had observable respiration but there was no significant difference in respiration rates, contrary to the hypothesis that aquatic microbial respiration is a function of organic sedimentation. Nonetheless, this organic sediment, could play other roles in shaping the microbial communities and in turn influencing other environmental factors, relevant to trout aquaculture

*Key words:* aquaculture, dissolved oxygen, microbe, sedimentation

### INTRODUCTION

Microbes are consequential for abiotic conditions and have important biotic interactions. For instance, microbes perform essential ecosystem functions, such as degradation of dead material, pathogenesis of living material, integral symbioses and mutualisms with many Eukarya and creating bioactive compounds for use against other microorganisms.

Microbial processes are a crucial feature of aquaculture environments, with positive and negative impacts. For instance, microbes have been introduced into these environments to outcompete pathogenic microbes and improve fish survival (Verschuere et al. 2000). Conversely, bacterial respiration can be detrimental to aquaculture by converting oxygen to CO<sub>2</sub>. Bacterial respiration makes up over 30% of biological oxygen demand in some carbon-rich environments (Smith et al. 1973). Increases in organic carbon inputs can lead to increases in microbial respiration, which can reduce oxygen in aquatic ecosystems. This reduction can have physiological effects on fish, such as reducing mobility, limiting metabolic rates, slowing growth, and causing death (Davis, 1975).

To examine microbial effects on abiotic conditions in the context of aquaculture, I ran a series of experiments in a small-scale rainbow trout (*Oncorhynchus mykiss*) aquaculture operation at Cuerici Biological Station, in Costa Rica. This system contains trenches connected to one another in series by a group of pipes, allowing water to gravity flow throughout the operation. A previous study examined how dissolved oxygen (DO) decreased as it passed into the ponds later in series, postulating that the removal of dissolved oxygen was a consequence of trout respiration (Wengert et al, 2009). A visual inspection of the initial influent indicated regular input of organic matter into the first pond. The flow patterns of the trenches suggested the possibility of sequential settling of organic matter. I tested the prediction that organic matter is greatest in the upper ponds. Organic input is a potentially important carbon source for microbial respiration. I tested the prediction that microbial respiration would be greatest in the ponds with the highest levels of suspended organic material.

## METHODS

### *Site description*

A small-scale rainbow-trout aquaculture operation located at the Cuerici Biological Station in Costa Rica, which in part consists of four rectangular trenches connected through drainage pipes (Figure 1). The influent comes from a nearby river, and the effluent of the fourth pond goes to a larger pond. All water flow is controlled by gravity, and very large debris is prevented from flowing between ponds with a coarse mesh covering on the connecting pipes.

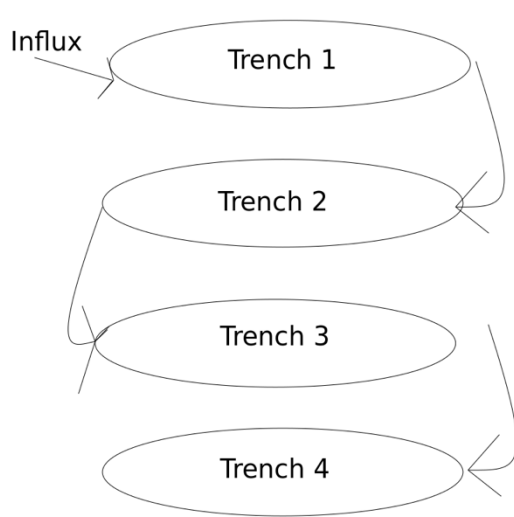


Figure 1: Diagram of the four study ponds. Individual ponds were long thin trenches (~30 m x 2 m), connected by pipes (represented by arrows in the diagram) such that water flowed between trenches by gravity.

### *Water column sediment analyses*

To test how particles settled along the ponds, I quantified the content of particles in the water column in each of the four ponds. I took water column samples from mid water column, as close to the center of the pond as possible. I decanted five liters of water and suspended particles into the center of a set of stacked sieves, with largest mesh size being 4000  $\mu\text{m}$  and smallest mesh size being 63  $\mu\text{m}$ , with 6 total size fractions. I rinsed the sieves to allow sediments to settle to the smallest possible size fraction. To quantify particles of sizes smaller than 63  $\mu\text{m}$  I saved all effluent. I separated size fractions onto filter paper and suspended in the air to dry. Then I recorded the dry mass of sediments from each size fraction.

### *Microbial respiration incubations*

I performed incubation experiments in triplicate for all four trenches using 1-L transparent screw top bottles filled with 800 mL of water from a pond and 25 grams wet weight of sediment. I created an abiotic control using 800 mL water from a potable source and no sediment. I measured dissolved oxygen concentrations every 30 minutes for 1.5 hours (i.e., four measurements), using a YSI Dissolved Oxygen Meter inserted into the bottle at each sampling time. To estimate potential effects from changing water temperature changes, I also measured temperature. Rates were calculated as the difference of dissolved oxygen concentrations between each time step divided by the sampling time (providing three rates for each replicate).

### *Flow rate*

I attempted to measure flow rate by fitting a known size container under the outflow of the pipes that connect the trenches and recording the time it took to fill. Due to the high water level of the trenches, this proved to be impractical, but there was no other visible flow in or out of any trench, so the flow rate must be consistent between trenches, save for whatever volume may leach into nearby soils.

### *Statistical Analyses*

All statistical analyses were performed in JMP Pro 13. To examine the relationship between dissolved oxygen and incubation time, I fit a linear regression of dissolved oxygen as a function of time for each trench. To determine if temperature of incubations was affecting solubility of dissolved oxygen and confounding the results of the incubation, I fit a linear regression with dissolved oxygen as a response to temperature for each trench, and there was no significant trend. I tested for differences in respiration rates among trenches with a nested ANOVA that included trench and bottles within trench as a random effect (4 trenches x 3 bottles per trench x 3 measurements per bottle). The abiotic control was not included in the ANOVA model.

## RESULTS

### Water column sediment analyses

Total sediment mass, especially the larger size fractions, decreased from first to last trench in the system, indicating that most of the sediments settle in the first or second trench (Figure 2).

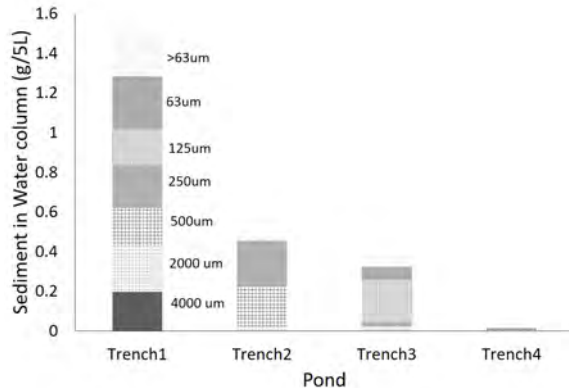


Figure 2: Sediment fractions extracted from 5 L of water from the mid water column for each of the four trenches.

Microbial respiration was evident as a linear decline in dissolved oxygen during incubations (Trench 1: slope  $\pm$  SE =  $-9.11 \pm 1.66$ ,  $P = 0.0003$ ,  $r^2 = 0.75$ ; Trench 2: slope  $\pm$  SE =  $-3.23 \pm 1.44$ ,  $P = 0.05$ ,  $r^2 = 0.34$ ; Trench 3: slope  $\pm$  SE =  $-8.91 \pm 1.71$ ,  $P = 0.0004$ ,  $r^2 = 0.73$ ; Trench 4: slope  $\pm$  SE =  $-10.40 \pm 2.64$ ,  $P = 0.003$ ,  $r^2 = 0.61$ ). As expected, dissolved oxygen did not decline during incubation in the abiotic control (slope  $\pm$  SE =  $-1.35 \pm 1.14$ ,  $P = 0.26$ ,  $r^2 = 0.12$ ) (Figure 3).

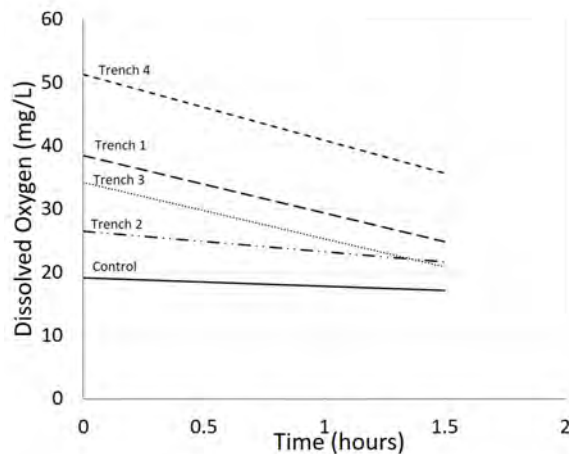


Figure 3: Average dissolved oxygen by time for incubations of water collected from all four trenches and abiotic control.

Respiration rates from trenches averaged  $4 - 11 \text{ mg DO} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ , which was well above that of tap water (Fig. 4). The ANOVA revealed significant variation in respiration rates among the 4 trenches ( $F_{3,8} = 5.11$ ,  $p = 0.029$ ), which was largely attributable to trench 2 having respiration rates that were less than half that of the other 3 trenches.

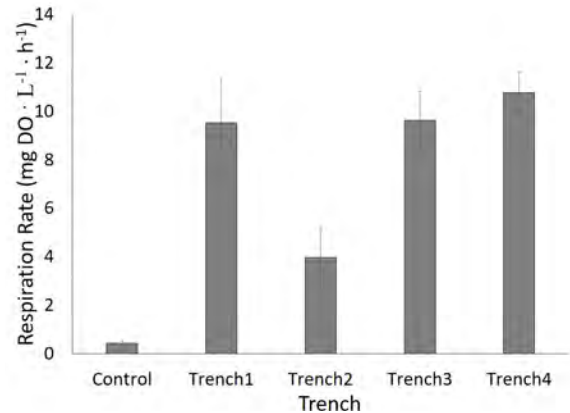


Figure 4: Average respiration rate by trench (mean  $\pm$  1 S.E.)

## DISCUSSION

My findings were consistent with the hypothesis that meaningful microbial respiration is occurring in this aquaculture system. The incubations proved that dissolved oxygen in these ponds is affected by microbial respiration. My findings were also consistent with the hypothesis that sedimentation is greatest in the first trenches. However, I did not find a difference in respiration rates between trenches, which was contrary to with the hypothesis that increases in suspended sediments cause increases in microbial respiration.

The ability for sediment particles to transport and change microbial communities has been previously documented, both through particle settling, (Duret et al. 2015) and through changing influent carbon richness in an environment. (Smith 1973). These previous studies suggest that although there was no effect of sedimentation on respiration rates, sedimentation can cause changes to the microbial communities that can cause shifts in microbial processes that impact the system. Examples of possible impacts include the oxidation of ammonia or nitrates from fish waste, competition against pathogens, and

production of bioactive metabolites. Screening for these types of impacts can be done by molecular analyses of the community composition, along with tests to determine community activity and effects on aquaculture.

Microbial communities can have profound impacts on their environments, but these impacts are not always easy to identify. While microbial communities can shape environments, environmental conditions also determine the composition of microbial communities that can survive there. These interactions make for a complex interplay of microbes and their environment, which can also affect other biota. In aquatic environments, these effects can be as small as changes in water quality modification in small-scale aquaculture operations or as large as changes in global sea-surface processes which may control large amounts of global carbon cycling.

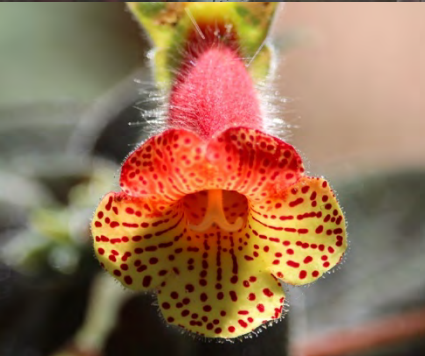
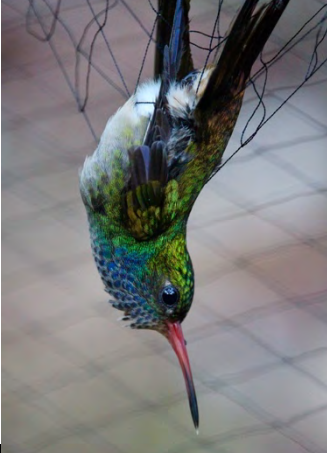
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#### LITERATURE CITED

- Davis, J. C. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on canadian species: a review. *Journal of the Fisheries Research Board of Canada* 32 2295-2332
- Duret, M. T., Pachiadaki, M. G., Stewart, F.J., Sarode, N. Christaki, U. Monchy, S., Srivastava, A., Edgcomb, V.P. Size-fractionated diversity of eukaryotic microbial communities in the Eastern Tropical North Pacific oxygen minimum zone. 2015. *FEMS Microbiology Ecology* 91. doi: 10.1093/femsec/fiv037
- Smith, K. L., Rowe, G. T., Nichols, J. A. 1973. Benthic community respiration near the woods hole sewage outfall. *Estuarine and Coastal Marine Science* 1, 65-70.
- Verschuere, L., Robaut, G., Sorgeloos, P., Verstraete, W. 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews* 64, 655-671.
- Wengert, S. E., Dashevsky, M. N., Wachter, J. M., Meyers, R.M. Susman, D. L. 2009. Trout hatchery water quality monitoring: a baseline study. *Dartmouth Studies in Tropical Ecology* 2009, 111-114.







## RIGHT OFF THE BAT: SEQUENCE OF CAVE EXITS AND CALL DYNAMICS IN A MULTI-SPECIES BAT COLONY

ALEXANDER W. COTNOIR, GRACE S. CALLAHAN, AND PAUL J. VICKERS

Teaching Assistants: Michael B. Brown and Debora Goedert  
Faculty Editor: Matthew P. Ayres

**Abstract:** Multi-species assemblages can create both interspecific and intraspecific competition for resources. Still, such groupings are a common occurrence. We examined how three species of insectivorous mormoopid bats, *Pteronotus parnellii*, *P. personatus*, and *P. gymnonotus* coexist within a large colony in Osa Peninsula, Costa Rica. Through analysis of acoustic recordings and net capture data during nightly exodus from the cave mouth, we found that species exited the cave in a distinct temporal pattern. We found no variation in cave exit sequence among species based on sex or reproductive status. Niche partitioning among mormoopid bats extends to acoustic space; our study species called at different frequencies, which largely eliminated the chance of jamming. Conspecific pairs did not appear to adjust their calls in periods of high bat activity to avoid intraspecific jamming.

**Key words:** acoustic partitioning, bats, coexistence, echolocation, group dynamics, Mormoopidae, niche partitioning

### INTRODUCTION

Group living is found across a range of species, from honeybees to humpback whales. Living in groups can benefit individuals through protection, aid in offspring care, food acquisition, and thermoregulation. Group living also comes with costs, such as increased disease transmission, competition for space and food, and greater competition for mates. Group living dynamics become more complex in groups of multiple species that consume a scarce resource. Interspecific competition is expected to be most intense among species that use similar resources. Niche theory suggests that for similar species to persist in such close proximity to each other, they must occupy slightly different roles and positions within a system (Hardin 1960).

Due to their communal living and common division of spatial, temporal, and acoustic space, bats provide a convenient system to study niche-partitioning. Shared caves are well-studied in temperate bats, but less is known about behaviors in multi-species bat colonies in the Neotropics (Mancina et al. 2012). Partitioning of acoustic space necessary for bat navigation has gained particular attention in recent years. Calls of surrounding bats can interfere with an individual's echolocation through a phenomenon known as jamming, but this has been little studied in multispecies cave colonies (Mancina et al. 2012).

Cave exiting time could vary among cohabiting bat species due to (1) preferred prey resources being available at different times, (2) different hunting methods requiring different light levels, or (3) prevention of jamming among echolocation calls. Temporal partitioning exits could also be the result of different species roosting in different places within a cave.

We analyzed inter and intraspecific variation in cave exit patterns and acoustic niche partitioning in an assemblage of Neotropical bats. We tested for temporal niche-partitioning across species by examining trends in cave emergence of three species of bat of the Mormoopidae family (mormoopids) inhabiting a single large coastal cave on the Osa Peninsula, Costa Rica. The three species - *Pteronotus parnelli*, *P. personatus*, and *P. gymnonotus* - are small, insectivorous bats that roost as large colonies, preferably in large caverns. Multispecies roosts of Mormoopidae bats are a common occurrence, but the causes and consequences are little understood (Bonaccorso 1979). Although these bats have similar diets, differences in exit time could correspond to slight variations in preferred hunting time that may be dictated by predation tactics. We tested whether species exit the cave in a predictable sequence after sunset.

We evaluated the effects of reproductive status and sex on cave emergence time within

each species. Reproductive females might tend to exit the cave first to meet their increased energy needs. On the other hand, predators may congregate at the mouth of the cave due to the daily consistency of bat exit, making it more dangerous to leave the cave first.

Additionally, we tested for acoustic partitioning within and among species. We evaluated the hypothesis that bat species will use echolocation calls at different frequencies, both to identify the calls of conspecifics and to avoid jamming between species. Jamming could also occur within species. One way to reduce jamming is for individual bats to alter their calling frequency when conspecifics are calling.

## METHODS

### *Study site*

We conducted our study at the entrance to a large multi-species bat cave facing the Pacific Ocean on the Osa Peninsula, Costa Rica. The cave was located approximately 1 km southeast of the Campanario Biological Station and was inhabited by at least three species of the Mormoopidae family, *P. personatus*, *P. parnellii*, and *P. gymnonotus*.

### *Net capture*

We captured bats exiting the cave at sunset on 29, 30, and 31 January 2018. We deployed a net (1.83 m x 1.22 m) at chest height in the most-traveled flyway of bats funneling out from the cave entrance. We began netting after the first bat exited the cave and then captured and identified bats for the next hour. For each captured bat, we recorded the time of capture, species, sex, and reproductive status (if female) as reproductive (lactating or pregnant) or non-reproductive. We identified lactating females by distinctive hair loss around enlarged nipples and pregnant bats by a swollen, firm stomach. We designated the time when the first bat was captured to be time “zero” for each night and recorded subsequent catch times as minutes after the first bat was caught. We used an ANOVA to test for effects of species on emergence time (data combined from all three nights). We analyzed the effect of sex on emergence time in *P. personatus* using a t-test. We tested for the effect of reproductive status (lactating or

pregnant) on female emergence time and tested for an interaction between reproductive status and species using a two-way ANOVA. We were unable to test the effect of sex on emergence time for *P. parnellii* and *P. gymnonotus* due to the low number of males captured, and we were unable to test the effect of reproductive status (lactating or pregnant) among *P. gymnonotus* because none were reproductively active.

### *Acoustic recording*

We recorded calls at the cave entrance over the course of the three consecutive nights while bats were being captured. We used an Avisoft UltraSoundGate 116Hn device, recording up to 250 kHz with a Condenser Microphone CM16. We began recording when the first bat was observed leaving the cave and continued recording for 45 minutes to 1.5 hours afterward. Due to technical difficulties, recordings from the second night were excluded.

### *Call identification*

We identified bat calls of the three species based upon distinct frequency and duration characteristics described in the primary literature (Bader et al 2015, Gooler 1987, Margarita and Miguel 2017) (Fig. 1). We selected a total of 30 call sequences for each of the three bat species; 20 instances where each bat species was observed calling alone (singles), and 10 instances where each bat species was calling alongside a conspecific (natural pairs). Given the density of bat calls within our recordings and the perceptiveness of bats to even small time durations, we designated single calls as those that were produced more than 0.04 seconds apart from the call of a conspecific (Bader et al 2015). We distinguished natural pairs as calls of conspecifics that were produced within 0.01 seconds of one-another. Because bats call at regular intervals, we used the interpulse interval as a way to assign calls to one individual. We examined variations in interpulse intervals to identify calls involving two bats, using a combination of timing, amplitude, and frequency features to recognize which of the calls corresponded to one bat or the other.

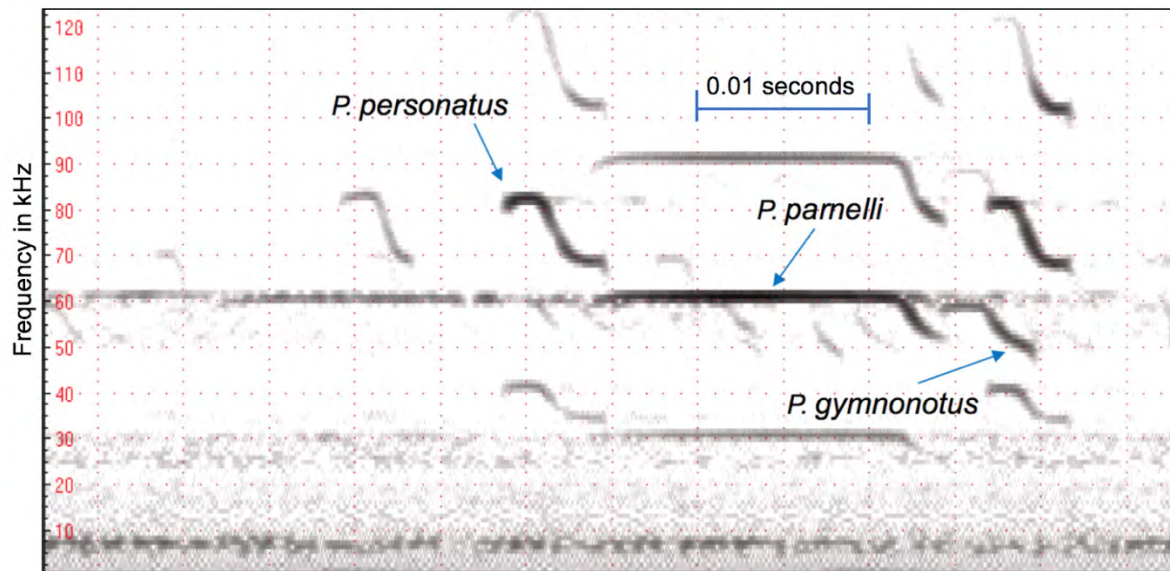


Figure 1. Call patterns and frequencies of *P. personatus*, *P. parnelli*, and *P. gymnonotus*. Arrows denote the dominant harmonic for each species. Note the comparatively long call of *P. parnelli* alongside the s-shaped calls of *P. personatus* and *P. gymnonotus*.

#### Call analysis

To test if echolocation calls exhibited greater variation in frequency between bats calling among conspecifics than bats calling alone, we compared the frequency of the harmonic with the most energy for natural pairs of calling bats with the frequencies of randomly paired calls of single bats. We created spectrograms on Avisoft UltraSound 6Tc to record the highest frequency of the harmonic with the most energy for each individuals' call. We recorded these frequencies for calls of the two individuals calling in 10 natural pairs, as well as for all 20 single-calling bats of each species. We calculated the difference in frequency between all ten pairs of bats that called within 0.01 seconds from one-another and compared this to the difference in frequency between all possible pairs of 20 randomly-selected bats that did not call within the same interval. We used t-tests to compare mean variance between single bat pairings and bats flying in natural pairs for each species.

We calculated and compared sound energy within the respective frequency ranges for *P. parnelli*, *P. personatus*, and *P. gymnonotus* calls. We first recorded the lowest and highest frequencies of the emphasized harmonic for 10

randomly-selected individuals of each species and averaged across the 10 individuals to calculate an average frequency range. Based upon these ranges, we designated calls within 46-58 kHz as *P. gymnonotus*, 58-62 kHz as *P. parnelli*, and 76-86 kHz as *P. personatus*. Using R v3.4.1 (R Development Core Team, 2013) and the packages “seeWave” (Sueur and Simonis 2008) and “tuneR” (Ligges et al. 2016) we calculated the total sound energy levels within each frequency band by summing total energy in each band over 10 second blocks for the duration of the recordings.

#### RESULTS

Over three nights, we captured 191 bats: 61 *P. personatus*, 123 *P. parnelli*, and 7 *P. gymnonotus* (Table 1). Females made up 57%, 99%, and 86% of captures *P. personatus*, *P. parnelli*, and *P. gymnonotus*, respectively.

Table 1. Number of males and females of each bat species captured on three nights.

Species	Total Males	Total Females	Reproductive Females	Non-reproductive Females
<i>P. personatus</i>	25	35	15	19
<i>P. parnelli</i>	1	122	108	14
<i>P. gymnonotus</i>	1	6	0	6

Cave emergence time differed between species (Fig. 2;  $F_{2,212}=54.76$ ,  $P<0.001$ ). *P. personatus* emerged significantly earlier than either *P. parnelli* or *P. gymnonotus* (Fig. 2;  $t=10.01$ ,  $P<0.001$ ,  $df=212$  and  $t=5.52$ ,  $P<0.001$ ,  $df=212$ ), but there was no difference in emergence time between *P. parnelli* and *P. gymnonotus* (Fig. 2;  $t=1.63$ ,  $P=0.10$ ,  $df=212$ ). Additionally, reproductive status (lactating or pregnant) had no significant effect on cave emergence times of *P. personatus* and *P. parnelli* females ( $F_{2,151}=1.12$ ,  $P=0.33$ ) and this result does not change between species (interaction term:  $F_{2,151}=1.52$ ,  $P=0.22$ ). For *P. personatus*, there was no significant difference between emergence times of males and females (Fig. 3;  $t=0.71$ ,  $P=0.48$ ,  $df=46$ ).

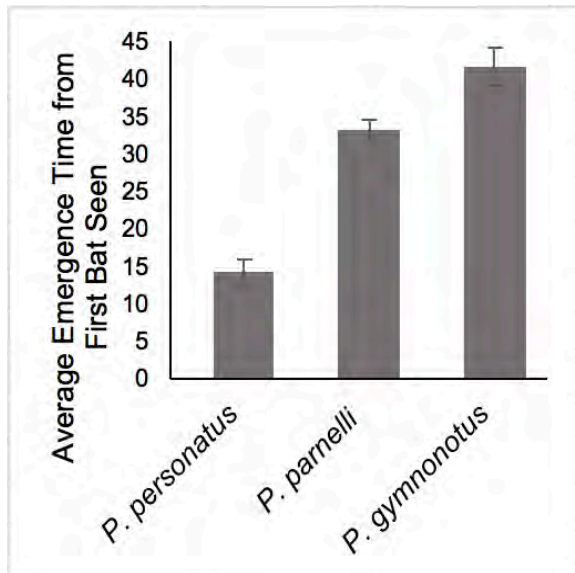


Figure 2. *P. personatus* emerged earlier than either *P. parnelli* or *P. gymnonotus*. There was no significant difference in emergence time between *P. parnelli* and *P. gymnonotus*.

Each bat species called at different frequencies (Fig. 1). The average frequency ranges of the emphasized harmonics for *P. personatus*, *P. parnelli*, and *P. gymnonotus* were found to be 81.53 - 68.65 kHz, 60.58 - 55.82 kHz, and 58.23 - 49.71 kHz respectively. Analysis of acoustic energy show that *P. personatus* echolocation bands contain the most energy for the first 22 minutes of each night, *P. gymnonotus* and *P. parnelli* bands contained approximately equal energy and both contained

more energy than *P. personatus* bands after the first 30 minutes of each recording (Fig. 4).

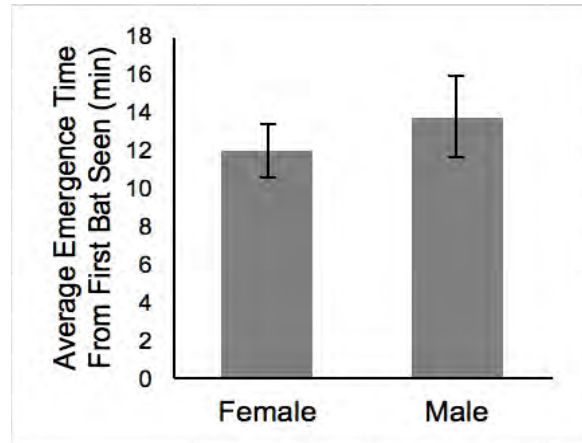


Figure 3. There was no difference between emergence times of *P. personatus* males and females ( $t=0.71$ ,  $P=0.48$ ,  $df=46$ ).

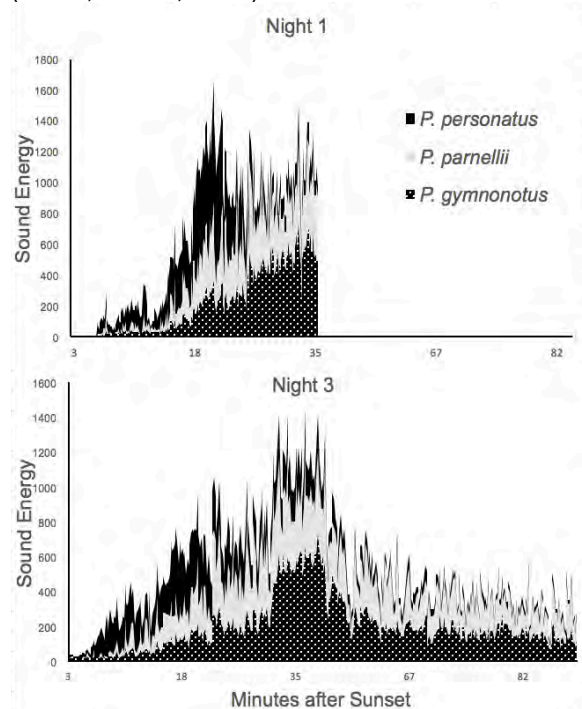


Figure 4. Relative sound energy recorded at echolocation frequencies of *P. gymnonotus* (46 kHz - 58 kHz), *P. parnelli* (58 kHz - 62 kHz), and *P. personatus* (76 kHz - 86 kHz) over 35 minutes in night one and 90 minutes in night three. Shading indicates proportion of total sound energy within the frequency range of each bat species. *P. personatus* was loudest during the first 1300 seconds. *P. parnelli* and *P. gymnonotus* were similarly prominent after that.

The presence of calling conspecifics had no detectable effect on call frequencies in any bat

species (Fig. 5; *P. gymnonotus*:  $t=0.02$ ,  $P=0.98$ ,  $df=198$ ; *P. parnelli*:  $t=0.30$ ,  $0.77$ ,  $df=198$ ; *P. personatus*:  $t=1.09$ ,  $P=0.28$ ,  $df=198$ ). The average difference in call frequencies between artificially- paired bats and natural pairs of simultaneous-calling bats across *P. gymnonotus*, *P. parnelli*, and *P. personatus* were only 0.02 kHz, 0.04 kHz, and 0.44 kHz respectively.

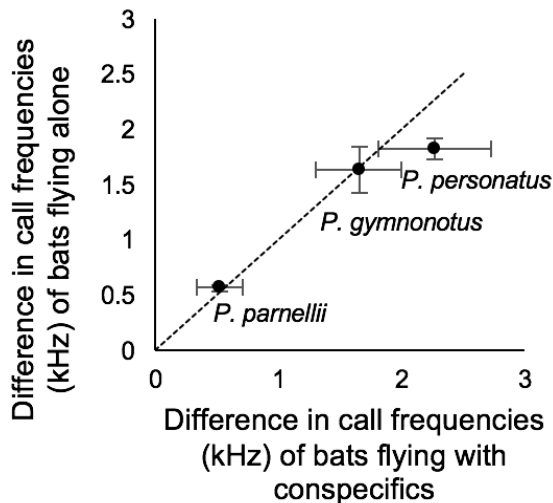


Figure 5. Bats did not displace calling frequencies in the presence of conspecifics. The difference in call frequency between bats that exited the cave together (x-axis) was indistinguishable from the difference in call frequency of random pairings of bats that exited the cave alone (y-axis). The line of equality represents no difference in call frequency. Error bars represent one standard error from the mean.

## DISCUSSION

The bat species in our study system varied in cave exit time and call frequencies, both of which could facilitate coexistence. Temporal partitioning of cave exiting sequence may be due to differences in body size (Villa-R. 1966) and feeding efficiencies (Mancina et al. 2012). The bat species within this assemblage differ not only in the frequency of calls emitted but in their mechanism of echolocation. *P. parnelli* is a “high-duty cycle” bat, meaning that they separate pulse and echo in frequency so that they can emit calls while simultaneously hearing echos (Brigham et al. 2002). As recent studies suggest, *P. parnelli* may not have to commence foraging activities as early in the evening when flight activity is highest, given their higher insect capture efficiency; which is enhanced by

their use of the doppler effect in prey detection (Mancina et al. 2012). Behavioral studies of multi-species colonies also demonstrate that smaller *Pteronotus* species are usually the first to exit a cave (Villa-R. 1966). This trend may be due to the fact that bats of smaller size are less easily captured by potential visual predators earlier in the evening, or that their feeding efficiencies are lower on average than larger species. Consistent with the size hypothesis, *P. personatus* are the smallest of the three species inhabiting the cave at our study site and exited the cave earlier than their cohabitants.

Reproductive status did not influence the order in which female *P. personatus* and *P. parnelli* exited the cave. Even though we could not test the effect of reproductive status on female exit time for *P. gymnonotus*, no females of this species we captured were reproductively active, perhaps indicating that non-reproductive species exit a cave later than reproductive species. To test this hypothesis, future studies must examine cave exit times over the course of the year to see if the exit times of *P. personatus*, *P. parnelli*, and *P. gymnonotus* change during their reproductive periods. If those species who are non-reproductively active leave the cave first throughout the year, it may provide evidence that bats are not exiting based upon their positions within the cave, but rather a drive to maximize foraging time during a particularly energy-intensive time.

Living in a large, multispecies colony could make effective echolocation, an essential ability for bat survival, difficult due to increased signal disruption (jamming) (Ratcliffe et al. 2004). Jamming only occurs between individuals of the same species, as each species has a distinct call frequency (Fig. 1). However, we found no evidence that individual bats of the same species will adjust their call frequencies in periods of high bat activity (Fig. 5), although this phenomenon has been observed in other species (Ratcliffe et al. 2004). However, we do not know the minimum kHz difference that these species are able to detect. Training bats to respond to the lower frequency call of two played recordings would allow researchers to determine the smallest difference in frequency bats are able to detect, providing information



about whether the small differences we observed are biologically meaningful. Additionally, previous studies that found evidence for bats altering their frequencies analyzed paired recordings from bats that were clearly flying alone. In our study design, we designated bats that called at least 0.04 seconds before and after any conspecifics as “singles”, although these may still have been hearing the calls of nearby bats, effectively reducing any change in frequency variation we would have expected to observe. Future investigations should analyze bat recordings at further distances from the cave entrance in areas of lower bat density to more definitively assess this question.

Our results provide insights into how large multi-species assemblages of mormoopid bats partition acoustic and temporal niches/space. Across three nights of data collection, we noted consistent trends in cave departure times between species, which may be attributable to differences among species in body size, prey hunting tactics, reproductive activity, spatial distribution with proximity to cave entrance, or a combination thereof. *Pteronotus parnellii*, *P. personatus*, and *P. gymnonotus* use disparate frequency bands when echolocating, effectively reducing the chances of a given species experiencing call jamming from individuals of other species. Thus, partitioning of acoustic space provides a possible explanation for how these species can cohabit caverns.

Understanding the mechanisms by which species-rich assemblages coexist may help identify caverns of particular importance to protect. Given the considerable role mormoopid bats play in energy flow within tropical systems and control of potentially harmful insect populations (Bateman and Vaughan 1974), conserving these animals is of particular importance in ensuring ecosystem stability. This research invites further study into the ways in which multi-species groups are able to live together, providing evidence that niche partitioning may extend beyond simple partitioning according to spatially-separate microhabitats and into acoustic space. Future examinations of multi-species groups, such as colonies of emperor, rockhopper, and gentoo penguins, may wish to examine cohabitation

through a multi-faceted lens, whereby factors such as food choice, hunting strategy, size, call type, etc. are examined simultaneously.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally to this study.

#### LITERATURE CITED

- Bader, E., Jung, K., Kalko, E. K. V., Page, R. A., Rodriguez, R., Sattler, T. 2015. Mobility explains the response of aerial insectivorous bats to anthropogenic habitat change in the Neotropics. *Biological Conservation*. 186: 97-106.
- Bateman, G. C., and T. A. Vaughan. 1974. Nightly activities of mormoopid bats. *Journal of Mammalogy*, 55: 45-65.
- Bonaccorso, F. J. 1979. Foraging and reproductive ecology in a Panamanian bat community. *Bull. Florida State Mus., Biological Sciences*, 24: 359-408.
- Fine, P. V. A. 2015. Ecological and evolutionary drivers of geographic variation in species diversity. *Annual Review of Ecology, Evolution, and Systematics*, 46: 369-92.
- Gooler, D. M. 1987. Topographic representation of vocal frequency demonstrated by microstimulation of anterior cingulate cortex in the echolocating bat, *Pteronotus parnellii parnellii*. *Journal of comparative physiology*, 161: 283-294.
- Herd, R. M. 1983. *Pteronotus parnellii*. *Mammalian species*, no. 209.
- Kalko, E. V., Handley, C. O., and Handley, D. CHAPTER 16 - Organization, Diversity, and Long-Term Dynamics of a Neotropical Bat Community, In *Long-Term Studies of Vertebrate Communities*, Academic Press, San Diego, 1996, Pages 503-553.
- Margarita, G. L., Miguel B.S. 2017. Composición y actividad de la comunidad de murciélagos artropodívoros en parques eólicos del trópico mexicano. *Revista Mexicana de Biodiversidad* 88: 888-898.

- Mancina, C. A., García-Rivera, L., and Bruce W. Miller. 2012. Wing morphology, echolocation, and resource partitioning in syntopic Cuban mormoopid bats. *Journal of Mammalogy* 93: 1308–1317.
- Ratcliffe, J. M., ter Hofstede, H. M., Avila-Flores, R., Fenton, M. B., McCracken, G. F., Biscardi, S., Blasko, J., Gillam, E., Orprecio, J., Spanjer, G. 2004. Conspecifics influence call design in the Brazilian free-tailed bat, *Tadarida brasiliensis*. *Canadian Journal of Zoology*, 82: 966-971.
- R Development Core. 2013. R: A Language and Environment for statistic computing. Vienna Austria: R Foundation for Statistical Computing.
- Salinas-Ramos, V. B., Herrera Montalvo, L. G., León-Regagnon, V., Arizabalaga-Escudero, A. and Clare, E. L. (2015), Dietary overlap and seasonality in three species of mormoopid bats from a tropical dry forest. *Mol Ecol*, 24: 5296–5307
- Sueur J., Aubin T., Simonis C. (2008). Seewave: a free modular tool for sound analysis and synthesis. *Bioacoustics*, 18: 213-226
- Uwe Ligges, Sebastian Krey, Olaf Mersmann, and Sarah Schnackenberg (2016). tuneR: Analysis of music. URL: <http://r-forge.r-project.org/projects/tuner/>.
- Villa-R., B. 1966. Los murciélagos de Mexico. *Inst. Biol., Univ. Nac. Auto. Mexico*, xvi + 492 pp
- Hardin, G. (1960). The Competitive Exclusion Principle. *Science*. 131: 1292–1297.

## MORPHOTYPES OF *ELEUTHERODACTYLUS* IN A COSTA RICAN TROPICAL FOREST

MAANAV JALAN, ANGELA E. ORTLIEB, MARY M. PEDICINI, BALTHASAR L. VON HOYNINGEN  
HUENE

Teaching Assistants: Michael B. Brown and Debora Goedert  
Faculty Editor: Matthew P. Ayres

**Abstract:** Organisms exhibit a wide variety of colors within and across species. The genus *Eleutherodactylus* includes over 600 species of neotropical terrestrial frogs and exhibits extreme polychromatism that is well-documented, yet poorly-understood. Our study explored the association of individual color variation with habitat selection and body measurements in a group of *Eleutherodactylus* around El Campanario Biological Station of the Osa Peninsula, Costa Rica. We initially classified our sample individuals into four morphotypes based on color patterns, and then (1) compared their abundances across spatial and temporal habitats, and (2) tested for association with body size and shape. We also examined the occurrence of secondary color traits with our chosen morphotypes. Color morphotypes did not vary in abundance by habitat type, did not differ in body size or shape, and showed only limited associations with secondary color traits. There was no evidence of ecological differentiation among color morphotypes.

**Key words:** ecotype, *Eleutherodactylus*, color variation, Osa Peninsula

### INTRODUCTION

Organisms within a population can display wide variations in color. Variations in coloration often afford ecological benefits to organisms, such as by increasing crypsis in certain habitats and thus allowing them to avoid predation. For example, Satyrinae butterflies have distinct summer and winter habitats, and exhibit stark dimorphism such that their summer and winter colorations match their seasonal habitat (Braby, 1994). In other cases, coloration performs no apparent ecological function, and does not influence an organism's fitness. Variegated squirrels, for example, display a large range of colors even within small geographical ranges, in spite of no clear adaptive function (Best 1995). As such, color morphotypes may or may not have ecological function in organisms.

Color polymorphism is frequently observed among frogs (order Anura), and notably in the neotropical genus *Eleutherodactylus*; this is the most speciose genus among vertebrates, containing about 600 species. Color polymorphism is widespread across species of *Eleutherodactylus*, with species presenting many different color morphs, while similar markings can occur in different species (Savage 2002). All color morphs appear cryptic, combining earth tones with visually disruptive markings

(Woolbright and Stewart 2008; Zuluaga 2017). Thus, coloration could have a general role in predator avoidance, with variations being specialized to match certain habitats leading to ecotypes.

To test for ecological correlates of color variation, we examined color polymorphism in *Eleutherodactylus* frogs around El Campanario Biological Station on the Osa Peninsula, Costa Rica. In this system, frogs of similar size, shape, habits and substrate (leaf litter) exhibit variations in color and pattern giving rise to several distinct morphotypes. We investigate if color morphotypes are unevenly distributed between two types of habitats: primary forest and forest edge (in day or night). If color variations represent ecotypes, morphotypes should be distributed non-randomly across habitats, with particular morphotypes present in higher number in one habitat than in others. Moreover, ecotypes can often evolve correlated variation in morphology; e.g., stickleback fish show distinct morphologies in marine and freshwater environments (Lavin and McPhail 1987). Similarly, frogs of similar morphologies are likely to be adapted to similar habitats. Therefore, we also evaluate if color morphotypes are associated with distinct body size and shape, i.e., morphology. If frogs of

different color morphotypes are found to have different morphological dimensions, we would have additional evidence that morphotypes represent ecotypes.

## METHODS

### *Morphotype classification*

Our preliminary surveys of *Eleutherodactylus* in the area suggested three common and distinct color patterns. Some frogs presented a characteristic black mask, others had white lateral stripes, while others had forelimbs golden in color with a patch of the same coloration at the tip of the snout. To test for ecological differences among frogs of different patterns, we created three morphotypes based on these visually striking characteristics: “Bandit”, “White Stripes”, and “Gilded” (Fig. 1). We grouped all frogs that did not fit into one of these categories as “Other”. Hereafter, we refer to color morphs by these morphotypes. For all frogs used in this study, we photographed the individual to document the color pattern for later classification into morphotypes.



Figure 1. Four *Eleutherodactylus* morphotypes: 1A. Bandit: 1B. White Stripes: 1C. Gilded: 1D.

### *Habitat type*

To assess distribution of morphotypes across habitats, we caught and measured 15 frogs from primary forest and forest edge during daylight

hours. To compare the daily cycle of activity across morphotypes, we caught an additional 15 frogs from secondary forest after sunset. In each habitat, we walked along manmade trails and looked for frogs in the leaf litter, capturing individuals by hand. We quantified proportion of morphotypes per habitat and performed a chi-square contingency analysis to determine if morphotype distribution differed non-randomly between habitats.

### *Morphology*

In at least one photograph of each frog, we included a ruler for scale. We used ImageJ software to obtain the following measurements for each frog: body width, snout-vent length (SVL), femur length, tibiofibular length, hind foot length, and front foot length (Fig. 2). We then classified each individual into a morphotype based on their most distinguishing color patterns.

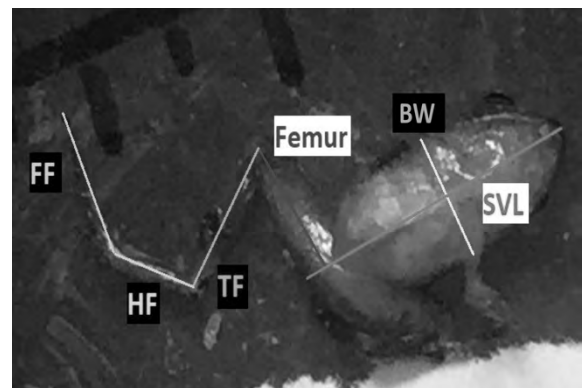


Figure 2. Measurements of body width (BW), snout-vent length (SVL), femur length (Femur), tibiofibular length (TF), hindfoot length (HF), and forefoot length (FF) as taken in ImageJ.

We performed a principal component analysis to consolidate physical measurements into characteristic values for each frog. Based on the loadings of each principal component, we interpreted PC-1 as body size and PC-2 as body shape. We then compared PC-1 and PC-2 to morphotype using a one-way ANOVA, then similarly compared PC-1 and PC-2 to habitat type. We performed all analyses in JMP Pro 13.0.

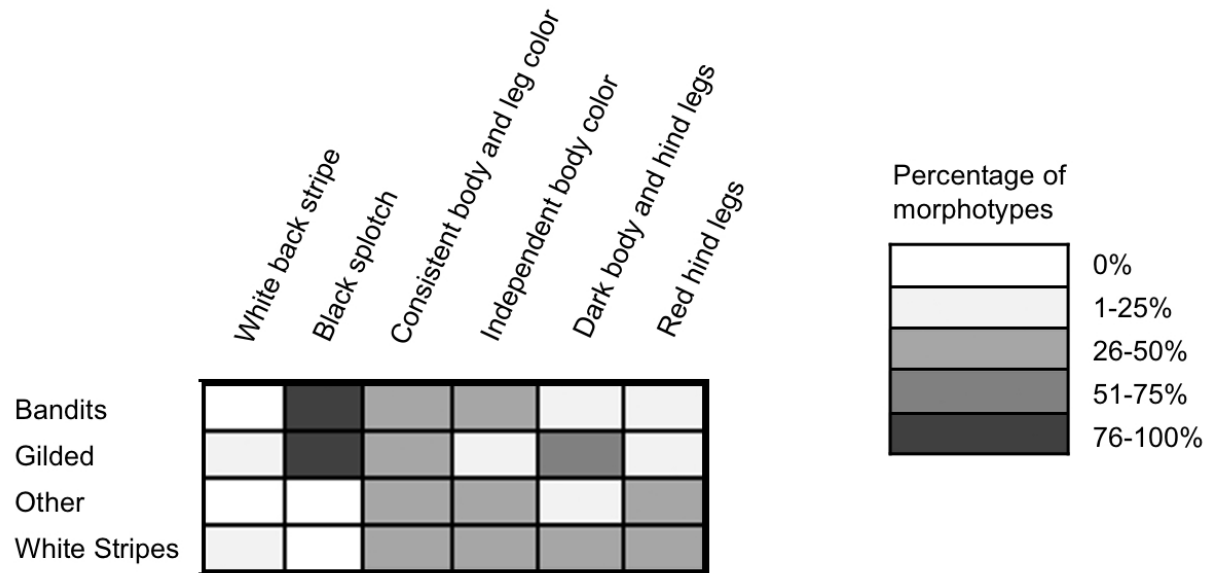


Figure 3. Percentage of morphotypes exhibiting six additional color patterns. The black spotch was prevalent in two morphotypes and absent in the other two.

#### *Additional color variation*

We categorized additional color variation across morphotypes by recording the proportion of individuals that presented identifiable color patterns within each morphotype (Fig. 3).

### RESULTS

#### *Morphotype classification*

Of the 45 frogs, only 4 were small (SVL 23 – 24.2 mm, sensu Savage 2002) while all others were tiny (9.6 – 20 mm). We excluded the small individuals from further analysis as they were a different type. The remaining 41 individuals were similar in size and body proportions and were all found among leaf litter (mean SVL  $\pm$  SE = 14.72 mm  $\pm$  0.41 mm), (mean weight  $\pm$  SE = 0.34 g  $\pm$  0.03 g).

#### *Habitat type*

All 4 morphotypes occurred with the same frequency across habitats and timescales (chi-square = 8.41,  $P = 0.21$ ,  $df = 6$ ; Fig. 4).

#### *Morphology*

The cumulative proportion of variance explained by the first two PC axes was 92.7% (PC-1 = 88%, PC-2 = 4.7 %; Table 1). The loadings of PC-1 were positive across body measurements, indicating that PC-1 represents body size. For PC-2, the loadings of SVL and body width were

positive, while all measures of hind leg length were negative. We therefore interpreted PC-2 as body shape, with higher values indicating more stout individuals with relatively shorter hind limbs. Neither body size nor shape differed significantly among the four morphotypes ( $F_{3,37} = 1.94$ ,  $P = 0.14$ ), ( $F_{3,37} = 0.96$ ,  $P = 0.42$ ). Further, frogs occupying different habitats did not differ in either size ( $F_{2,38} = 1.27$ ,  $P = 0.29$ ) or shape ( $F_{2,38} = 2.28$ ,  $P = 0.12$ ) suggesting that no habitats favored certain morphological characteristics.

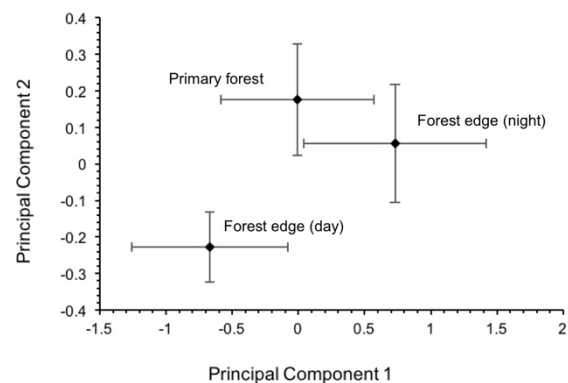


Figure 4. There were no significant differences among the three habitat types in frog size (PC-1) or frog shape (PC-2).

Table 2. PCA loadings of frog morphological measurements. Body measurement codes: BW = body width, SVL = snout-vent length, TF = tibiofibular length, HF = hindfoot length, FF = forefoot length

Body measure	Loadings	
	PC-1	PC-2
BW	0.9	0.41
SVL	0.97	0.15
Femur	0.94	-0.07
TF	0.97	-0.13
HF	0.91	-0.13
FF	0.93	-0.22
% Variance	88	5

#### Additional color variation

We identified 6 additional color variations within morphotypes: black side splotch, single white stripe down the center of the back, uniform body and leg color, distinct body color from legs, dark body and hind legs, and red hind legs. While certain secondary traits were exclusive to some morphotypes, others varied considerably within and across morphotypes. Trends across morphotypes include the absence of black splotches in Bandits and White Stripes and a prevalence of black splotches in Gilded and Other. Also, middle white stripes were absent in Other and White Stripes, while commonly present in Bandits and Gilded (Fig. 3).

#### DISCUSSION

*Eleutherodactylus* morphotypes in our system appeared to be ecologically redundant, as they co-occurred across habitats and time of day. Additionally, coloration did not correlate with other morphological features such as body size and shape. As such, color morphotypes seem to reflect genetic variation not associated with ecotype differentiation in *Eleutherodactylus*. Additionally, we observed additional color patterns beyond those used to define morphotypes, such as black splotches, red legs, and a single white stripe down the center of the back (Fig. 3). Most color patterns occurred along a gradient throughout the sample population,

which could be a product of interbreeding, or variability inherited from a common ancestor. This observed polychromatism could have arisen from apostatic selection on the population as a means of disrupting predators' search image (Woolbright and Stewart 2008). Our results suggest the possibility that color morphotypes represent variation within a single species, although further studies would need to investigate reproductive behaviors in this system to verify such prediction.

The different morphotypes may be specialized to smaller-scale habitats than those we examined. For example, Gilded frogs might camouflage well in patches of forest floor spotted with diffuse sunlight, whereas Bandits may be more suited to shady areas, beneath leaf litter or logs. Further studies should investigate the relationship between coloration and habitat on a microhabitat scale. Since we were unable to identify the individuals in our study to the species level, it is possible that our sample contained multiple species of *Eleutherodactylus*. Future studies should attempt to identify the species present in our system. The absence of any correlation between morphotype, habitat type, and body size and shape suggest that if these individuals do indeed belong to different species, species distinctions are upheld through mechanisms we could not assess.

Considering the possibility that morphotypes are distinct species that occupy the same ecological niches, there may be non-visual sensory cues that restrict gene flow and impose reproductive isolation, and it is possible that color works as a mechanism for species recognition. Gomez et al. (2009) showed that coloration influences the mating behaviors of certain frog species, such as the European tree frog (*Hyla arborea*). Yet, frog visual systems are less light-sensitive than human visual systems, with a single layer of ganglion cells that specializes in detecting motion rather than intricate details (Lettvin et al. 1959). As such, the color patterns that define the morphotypes in this study may be too subtle for frogs to perceive. A longer-term study could investigate the *Eleutherodactylus* sensory system and mating behaviors in the context of sexual selection pressures on speciation. For example,



for organisms that rely heavily upon species-specific calls to identify and locate mates, subtle differences in mating calls can isolate populations and even define species; e.g., greenish warblers distributed around the Tibetan Plateau diverge into distinct species despite close proximity based upon differences in male songs (Alcaide et al. 2014). In this case, color variation could be the result of genetic differentiation across species or incipient species of tiny *Eleutherodactylus*.

Our study was relevant to broad questions about the significance of color variation within closely related organisms. In this group of *Eleutherodactylus* frogs, color variation does not indicate ecotype in the context of morphological variation or habitat specialization. The observed coloration gradient raises the possibility that this group is a panmictic population with considerable polychromatism resulting from any number of factors. If these *Eleutherodactylus* do indeed represent different species, the species distinctions are upheld not by color, habitat specialization, or morphology, but rather another mechanism that has yet to be investigated. On a broader scale, color variation can have multiple causes and effects in a group of individuals. Evaluation of such variation can offer insight into the complexities that inform species distinctions.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

- Alcaide, M., E. S. C. Scordato, T. D. Price, and D. E. Irwin. 2014. Genomic divergence in a ring species complex. *Nature* 511: 83-85.
- Best, T. 1995. Mammalian species: *Sciurus variegatoides*. *The American Society of Mammalogists* 500: 1-6.
- Braby, M. F. 1994. Phenotypic variation in adult *Mycalesis hubner* (Lepidoptera: Nymphalidae: Satyrinae) from the Australian wet-dry tropics. *Australian Journal of Entomology* 33: 327-336.
- Gomez, D., C. Richardson, T. Lengagne, S. Plenet, P. Joly, J. Léna, and M. Théry. 2009. The role of nocturnal vision in mate choice: females prefer conspicuous males in the European Tree Frog (*Hyla arborea*). *Proceedings: Biological Sciences* 276: 2351-2358.
- Lavin, P. A., and J.D. McPhail. 1987. Morphological divergence and the organization of trophic characters among lacustrine populations of the threespine stickleback (*Gasterosteus aculeatus*). *Canadian Journal of Fisheries and Aquatic Sciences* 44: 1820-1829.
- Lettvin, J. Y., H. R. Maturana, W. S. McCulloch, and W. H. Pitts. 1959. What the frog's eye tells the frog's brain. *Proceedings of the IRE* 47: 1940-1951.
- O'Neill, E. M. and K. H. Beard. 2010. Genetic basis of a color pattern polymorphism in the Coqui frog *Eleutherodactylus coqui*. *Journal of Heredity* 101: 703-709.
- Savage, J. M. 2002. *The Amphibians and Reptiles of Costa Rica*. The University of Chicago Press, Chicago.
- Woolbright, L. L. and M. M. Stewart. 2008. Spatial and temporal variation in color pattern morphology in the tropical frog, *Eleutherodactylus coqui*. *Copeia* 2008: 431-437.
- Zuluaga, R. B. 2017. Behavioural, ecological, and evolutionary aspects of diversity in frog colour patterns. *Biological Reviews* 92: 1059-1080.

## SHELL FIT INFLUENCES AGGREGATION PATTERNS OF HERMIT CRABS

ALEXANADRA M. STENDAHL, CORINNE R. VIETORISZ, CLAYTON E. JACQUES

Teaching Assistants: Michael Butler Brown and Debora Goedert

Faculty Editor: Matthew P. Ayres

*Abstract:* Individuals may be more or less likely to join aggregations depending on costs and benefits to their fitness. Hermit crabs aggregate around both food and shells. We tested whether the fit of crabs to their shell was related to their tendency to aggregate around food versus empty shells. Hermit crabs aggregated around food had shell fit that matched randomly sampled crabs, whereas crabs aggregated around empty shells had relatively small shells for their body size. Shell fit did not vary with the size of the crabs. Crabs in shell aggregations only gathered around shells larger than their body size. Crabs seemed able to assess their shell size and fit and join aggregations that matched their needs.

*Key words:* aggregation, *Coenobita compressus*, fission-fusion, foraging, hermit crabs, shell fit, social behavior

### INTRODUCTION

Many animals spend time as singletons and occasionally fuse into conspecific groups depending on the environment and behavior. The benefits of group aggregation include reduction in predation risk and benefits from social relationships (van Schaik 1999). However, grouped individuals may also compete more intensely for resources such as food or shelter. Natural selection should favor individuals that behave to minimize the costs of competition and maximize the benefits of aggregation.

Resource distributions can strongly affect fission-fusion dynamics and group aggregation. Spotted hyenas (*Crocuta crocuta*) live in large social groups but often fission and forage in smaller parties to increase the share of prey for each individual in the party (Smith et al. 2008). However, when food resources are patchy, groups may aggregate. Aplin et al. (2012) found that 3 tit species (birds in the family Paridae) utilize social information to find and group around isolated food patches. The positioning of resources influences the way individuals form groups or disperse to acquire them.

Hermit crabs (*Coenobita compressus*) represent an attractive system to study fission-fusion dynamics because crabs compete for different kinds of resources: shells and food. Food provides energy for growth and reproduction, and shells are valuable, transportable shelters that require remodelling to

improve inhabitability (Laidre 2012). Shells that are too large potentially inhibit crab activity, and shells too small do not adequately protect the hermit crab's body. Growing hermit crabs generally seek larger shells to accommodate and protect larger body sizes. Aggregations of hermit crabs form around both shell and food resources, likely because early discoverers inadvertently transmit location information to conspecifics; groups do not appear to form to dilute predation threats (Laidre 2010). The size of individuals' shells and shell fit may influence their aggregation behavior to join groups around shells or food.

Our study investigated social aggregation behaviors in a hermit crab population with variation in shell fit. We hypothesized that hermit crabs join aggregations around different resources because of individual needs. Hermit crabs should join aggregations around resources they require (e.g., food or empty shells of a specific size) and will not join aggregations around unnecessary resources. Alternatively, crabs may join aggregations automatically, regardless of their need for the resource. If hermit crab aggregation patterns relate to shell fit, we predict that hermit crabs attending aggregations around empty shells will have smaller shells relative to body size than foraging or randomly sampled individuals. If hermit crabs recognize and compare shell sizes, we predict: 1) crabs will attend aggregations around shells larger than their own shell and 2) larger crabs

will aggregate around larger shells. To assess how shell fit varies across the population, we also estimated population size and investigated shell fit by crab size.

## METHODS

### *Population Counts*

This study was conducted in the Osa Peninsula of Costa Rica at El Campanario research station. Hermit crabs were sampled within 1 km of the research station. We defined three areas of beach for our study of population estimates: Red, Yellow, and Blue Beaches (Figure 1). A freshwater stream separated Red and Blue Beaches, and large rocks separated Red and Yellow Beaches. We marked the shells of 77 hermit crabs each on Red and Blue Beaches with red and blue nail polish, respectively. We also marked the shells of 81 more crabs on Yellow Beach with yellow nail polish. We surveyed the population of Red Beach 24 and 48 hours after marking and the populations of Blue and Yellow Beaches 24 hours after marking. In all cases, we walked the beaches while noting the number that had been marked.

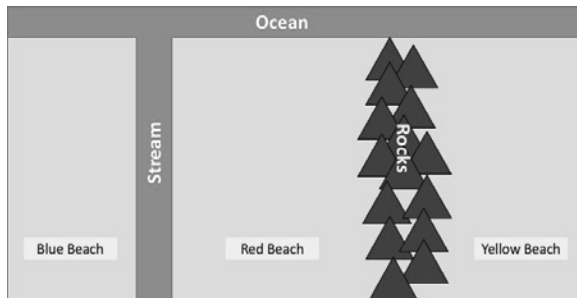


Figure 1. Map of beaches sampled.

To estimate the beach population sizes, we used a Lincoln-Peterson mark-recapture model:

$$\frac{n}{m} = \frac{N}{M} \quad \text{Equation 1}$$

where M represents the number of crabs marked, n the number of crabs captured during the subsequent survey, and m the number of recaptured crabs (previously marked). We solved for N, the size of the population.

### *Shell Fit Comparisons*

To test whether shell fit influences social aggregation dynamics, we measured the size of the hermit crabs and the size of the associated shells in three subpopulations of crabs: (1) those aggregated around food, (2) those aggregated around an empty shell, and (3) a random subset of crabs from the beach. We created the subpopulations aggregated around food by placing pieces of pineapple and papaya fruit on the beach and returning 15 minutes later. We attempted to similarly create aggregations around empty shells, but no aggregations formed. Instead, we located two naturally occurring aggregations, each concentrated around one empty shell. We then selected a random set of crabs by walking two 10-meter long transects that were parallel to, and 5 meters below, the high tide mark (one transect each on the Red and Yellow Beaches). We walked each transect several times, measuring all crabs within 1 meter of the transect.

We measured crab size by gently pulling the animal halfway out of its shell to expose the thorax and recorded the width of the widest part of the thorax with a pair of calipers (Figure 2a). We then allowed the crab to withdraw inside its shell and measured across the opening of the shell (Figure 2b).

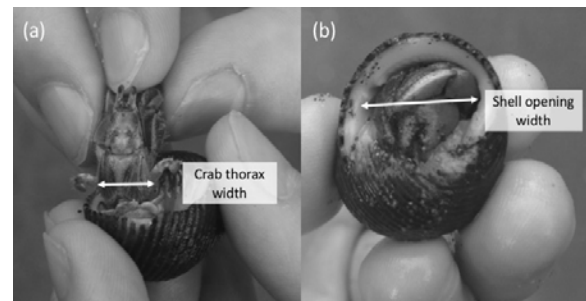


Figure 2. Methods of measuring crab size (a) and shell size (b).

To examine the relationship between body size and shell size across the three aggregation types, we first used linear regressions to compare the three aggregation types with respect to the relationship between size of crabs and the size of their shell. Following Neter et al. (1985, compare regressions), we evaluated the null

hypothesis that the slope and intercept of all three subpopulations was the same.

To compare the crab sizes in each aggregation around an empty shell, we plotted the sizes of all of the crabs in each aggregation. To calculate the approximate size of the crab that would occupy the empty shell that the aggregation was centered around, we used the regression equation of shell fit from the randomly sampled crabs:

$$\text{Shell size} = 1.68 + 1.34 \times \text{Crab Size} \quad \text{Equation 2}$$

We then compared the sizes of the crabs in that aggregation to the calculated crab size of the empty shell. All statistical analyses were conducted in JMP 13.0 Pro and Microsoft Excel 2016.

## RESULTS

### Population Counts

Our population size estimates from the mark-recapture study ranged from 7,929 to 22,275 hermit crabs (Table 1). We also saw intermixing of the populations despite the natural barriers, as we recaptured 6 marked crabs on different beaches than where they were originally marked (1 blue on red beach and 5 red on yellow beach).

Table 1. Estimates of hermit crab populations on three separate beaches by marking and observation.

Beach Color	Date	Initial Captures		Captures one day later		Population Estimate
		Number of Crabs Marked	Total	Recaptures		
Red	30-Jan	77	1397	11		9779
Blue	30-Jan	77	725	7		7925
Yellow	1-Feb	81	1100	4		22275

### Crab Size vs. Shell Size

We observed aggregations of 15 and 12 crabs around empty shells. They included a range of crab sizes (mean + SD = 2.80 mm + 1.48) and shell sizes (mean + SD = 4.33 + 1.82). The food aggregations included 19 and 44 individuals of various body sizes (mean + SD = 3.52 + 0.91) and shell sizes (mean + SD = 6.76 + 1.59). In our random transects, we counted 22 and 19 individuals of various body sizes (mean + SD = 3.20 + 0.84) and shell sizes (mean + SD = 5.99 + 1.35).

The relationship between shell size and crab size differed among the three aggregation types

(Figure 3;  $F_{4, 125} = 13.32$ ,  $P < 0.001$ ). The groups of crabs aggregated around shells had smaller shell sizes given their size than groups aggregated around food, or a random sample of crabs from the beach. The crabs aggregated around food were indistinguishable from the random sample of crabs.

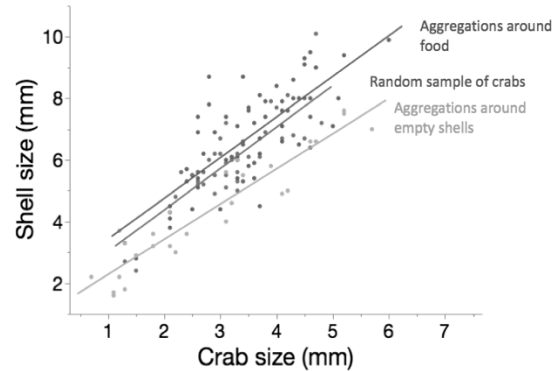


Figure 3. Shell size as a function of crab size by aggregation type. Each point represents a single crab that was found in an aggregation around a piece of food, in an aggregation around an empty shell, or sampled randomly across the beach.

Examination of the regression residuals for the randomly selected crabs in Figure 3 did not reveal any conspicuous patterns of higher or lower variance in the residuals at any crab size (Figure 4).

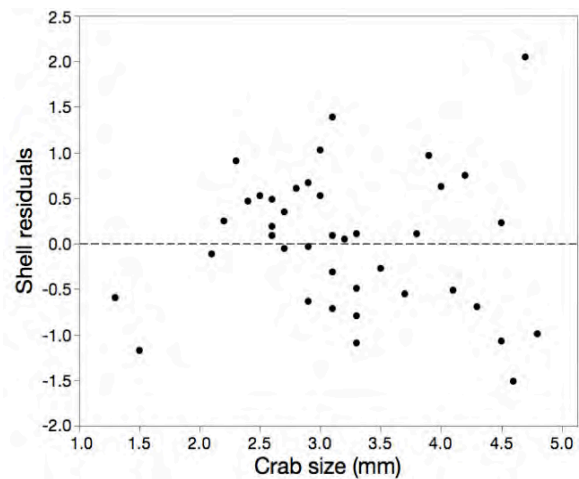


Figure 4. Residuals of shell fit as a function of crab size. Residuals are shown only for crabs sampled randomly. Residuals were from the regression line for randomly sampled crabs from Figure 3.

The crabs aggregating around a shell with a shell opening of 5.1 mm were smaller in body size than the crabs aggregating around a shell with an opening of 14.2 mm. The sizes of crabs in the aggregation around the 14.5 mm shell were significantly larger than the sizes of the crabs in the aggregation around the 5.1 mm shell ( $t = 8.50$ ,  $P = <0.001$ ,  $df = 19$ ). With the exception of one crab (3.1 mm thorax) in an aggregation around an empty shell (5.1 mm opening), all the crabs aggregating at empty shells were smaller than the estimated crab size of each respective empty shell (Figure 5). In every case, individual crabs had shells smaller than the empty shell at the center of the aggregation.

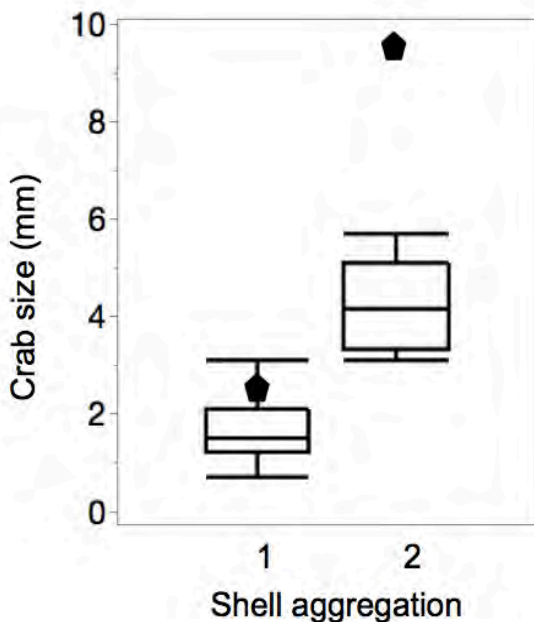


Figure 5. Sizes of crabs in two aggregations around empty shells. The two pentagons represent the calculated sizes of a crab that would match the empty shell size from Equation 2. Aggregation 1 was centered around an empty shell of 5.1 mm and Aggregation 2 around an empty shell of 14.2 mm.

## DISCUSSION

Our findings suggest that the shell fit of an individual influences the type of aggregation it is more likely to join. Crabs with small shells relative to their body size compared to a random sample of crabs on the beach were more likely to be aggregated around an empty shell. The fact

that the shell fit of crabs aggregating around food were indistinguishable from randomly selected crabs, while those aggregated around shells had relatively small shells, implies that crabs seeking shells are a modest subset of the total population compared to crabs that are attracted to food.

Our estimates of hermit crab population sizes seemed surprisingly large. This may help explain the consistent variation in shell fit across all crab sizes. We found approximately equal variance across crab sizes in the relationship between residuals of shell fit and crab size, suggesting the absence of any classes of shell size that were very rare compared to others (Figure 4). One explanation for the generally high fit of crabs to their shells is extensive trading of shells in the population. Crabs have a high likelihood of finding shells of the preferred size when crabs are constantly changing shells in a large population. The apparent lack of barriers to movement between beaches indicates these are not three separate populations, but rather one large population with over 40,000 individuals. More hermit crabs on adjacent beaches and walkways indicate that our population count is an underestimate and further population studies would be needed to get a more accurate estimate of total population size.

Our results are consistent with the primary hypothesis that a crab's shell influences aggregation behavior. Crabs appear to recognize how well their shell fits and use that information to decide whether or not to join a certain type of aggregation. Rather than joining an aggregation regardless of the resource type, they appear to specifically choose the aggregation they join based on the resource and their individual needs. In addition to awareness of shell fit, hermit crabs seem to recognize their own shell size in relation to other shells and perhaps individuals. Although we only found two aggregations around empty shells, all the crabs in both of the shell aggregations were smaller than the empty shell they were aggregating around, and all but one were smaller than the expected crab size that would fit the empty shell (Figure 5). Furthermore, the crabs aggregating around the smaller shell were significantly smaller than the crabs aggregating around the larger shell. This

trend shows that crabs are able to assess the size of empty shells in relation to the size of their own and join a group only when a shell (or the shell left behind by a crab transitioning into the new shell) could benefit them.

Further studies could provide more information about crabs' ability to gauge the size of shells, for example one could experimentally test crab preference for two differently-sized shells. One could also manipulate the fit of shells crabs' shell fit through shell replacements and test for effects on conspecific interactions and aggregation behavior.

Our findings indicate that hermit crab social behavior is influenced by their changing needs and self-awareness. These factors drive their fission-fusion social structure and group dynamics. The concept of social groups being influenced by the changing needs of individuals applies to many species outside of hermit crabs. All groups are composed of individuals with variable needs, and the changing states of the individuals causes changing states of their social structures. Social structures are not static, and understanding what factors influence their composition can help us gain insights into broader social dynamics of animal behavior.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

- Aplin LM, Farine DR, Morand-Ferron J, and Sheldon BC. 2012. Social networks predict patch discovery in a wild population of songbirds. *Proc R Soc B Biol Sci* 279: 4199–205.
- Laidre ME. 2010. How rugged individualists enable one another to find food and shelter: field experiments with tropical hermit crabs. *Proc R Soc B Biol Sci* 277: 1361–9.
- Laidre ME. 2012. Homes for hermits: temporal, spatial and structural dynamics as transportable homes are incorporated into a population. *J Zool* 288: 33–40.
- Neter, J., W. Wasserman, and M. Kutner. 1985. *Applied linear statistical models*. Richard D. Irwin, Inc., Homewood, Illinois.
- van Schaik CP. 1999. The socioecology of fission-fusion sociality in Orangutans. *Primates* 40: 69–86.
- Smith JE, Kolowski JM, Graham KE, et al. 2008. Social and ecological determinants of fission-fusion dynamics in the spotted hyaena. *Anim Behav* 76: 619–36.



## KATYDIDS OF DIFFERENT SIZES, SEXES, CLADES, AND COLORS VARY IN BEHAVIORAL RESPONSES TO CUES OF PREDATION RISK

ALEC B. COBBAN AND HANNAH I. HOFFMAN

Teaching Assistants: Michael B. Brown and Deb Goedert  
Faculty Editor: Matt Ayres

*Abstract:* Predators frequently drive organisms to evolve predation avoidance strategies. These behavioral sequences can be subtle and intricate, yet their patterns and causes are seldom studied. We test two competing hypotheses regarding antipredator strategies: (1) that responses to threatening stimuli are unpredictable to prevent predators from learning patterns and (2) that responses to threatening stimuli belong to larger behavioral syndromes, following predictable patterns based on one or more factors. We tested these hypotheses by exposing katydids across three subfamilies and diverse morphologies to two threatening stimuli and analyzing their subsequent responses. Individuals exhibited consistent behavioral sequences when exposed to a given stimulus, and patterns were found in sequences based on stimulus type, phylogeny, sex, and morphology. These results support the notion that antipredator strategies are not random but rather have likely evolved to suit their organisms in regard to predator type and natural history.

*Key words:* antipredator response, katydids

### INTRODUCTION

Prey often display evolved responses to predators that reduce risk and improve survivorship. While some species react simply and consistently to ranges of threats, others display intricate and variable behaviors drawn from a suite of possibilities. Due to this complexity, patterns in and causes for specific antipredator behaviors and sequences of behaviors are little understood for many groups of species. Two competing hypotheses emerge regarding antipredator responses in such individuals: (1) that reactions to threatening stimuli are variable such that predators cannot easily predict evasive maneuvers and (2) that responses to threatening stimuli reflect syndromes in which behaviors are correlated with the natural histories of individuals (Domenici et al. 2008).

Katydid provide a model system in which to test these hypotheses, as individuals within this family (Tettigoniidae) vary drastically in regard to natural history characteristics that could impact behavioral suites. We exposed katydids belonging to three subfamilies to stimuli and monitored their responses, classifying behaviors based on actions and antennae movement. We generated air flow to simulate flying, jumping, or otherwise quickly-

moving and relatively distant predators, and we used touch to simulate all other, relatively proximal predators. We subsequently determined how antipredator responses change across a variety of factors relating to predator type and natural history. Because a stimulus activates specific receptors on an individual, we hypothesize that individual katydids will exhibit the same behavior when exposed to a given stimulus (Fain 2003). Different stimuli can be attributed to predation situations that necessitate unique responses, and they also activate unique receptors; therefore, we hypothesize that individual katydids should exhibit distinct behaviors across the two stimuli (Fain 2003). Individuals with similar evolutionary histories are more likely to share behavioral suites, so we hypothesize that phylogeny will correlate with certain antipredator responses (Sih et al. 2004). Behavioral suites often develop over time; therefore, katydids in different life stages should exhibit antipredator strategy disparities (Tau & Peterson 2010). We hypothesize that antipredator strategy will vary based on sex, as male and female insects differ in several regards including parental care, mating behaviors, and hormone levels, all of which could initiate different responses to threats (Ryan 1998). For example, Lang et al. (2007) showed that female

katydids demonstrate site fidelity, so these individuals might be less likely to abandon their offspring when predators approach. Lastly, katydids that share morphological traits might occupy similar niches, and these traits might correlate with specific abilities; as such, these individuals should exhibit similar antipredator responses (Lardner 2000). Similarly, selection may act differently on morphologically-distinct katydids, resulting in a consistent expression of behavior based on physical characteristics.

## METHODS

### *Specimen sampling and general procedure*

We conducted nighttime surveys along trail networks at the Campanario Biological Research Station in Costa Rica to collect katydids from low vegetation. We also collected katydids from within a light trap (LED light in front of a white sheet) on the edge of the forest.

We evaluated katydid behavioral responses to simulated predator stimuli in an arena constructed from a white sheet. We placed an individual in the arena and waited for it to display normal resting behavior, as evidenced by lack of motion and oftentimes antennal cleaning. To simulate flying or quickly-approaching predators, we positioned a drinking straw roughly five centimeters directly behind the katydid and blew lightly to produce air flow. We repeated this stimulation twice more for each katydid, waiting after each event for it to display normal resting behavior. The following day, we repeated the procedure for the tactile stimulus. These trials consisted of touching the katydid with a blunt, wooden stick using the same pressure on its hind left foot, hind right foot, and back in a randomized order and waiting for it to display normal resting behavior after each response. We videotaped all trials for subsequent evaluation.

To score observed responses to stimuli, we developed a behavioral index based on a six-part response sequence: (1) an initial action of either freezing, flinching, walking, or jumping; (2) an initial antenna response of either no movement, slow movement, or fast movement; (3) an initial antennal directionality (if it had moved them) that appeared either random or directed towards the stimulus; (4) a secondary action of either

freezing or walking; (5) a secondary antennal response of either no movement, slow movement, or fast movement; and (6) a secondary antennal directionality (if it had moved them) that appeared either random or direction towards the stimulus. We analyzed the video footage for each trial using this index to quantify the behavioral responses exhibited. For each individual, we subsequently found the mode response for each stimulus, indicating the most repeated behavior for each of the six parts of the response sequences explained above.

### *Patterns related to stimulus type*

We evaluated possible associations between stimulus type and antipredator response by creating and examining data tables that compared the number of katydids that exhibited each of the possible alternative behaviors when stimulated with air flow or touch.

### *Patterns related to phylogeny*

We evaluated possible associations between subfamily and antipredator response by creating and examining data tables that compared the number of katydids in each subfamily (i.e., Pseudophyllinae, Agraeciinae, and Conocephalinae) that exhibited each of the possible alternative behaviors when stimulated with air flow or touch. The mode behavioral sequences of two same-species pairs for each stimulus were compared to investigate which factors (described below) could explain potential discrepancies even when genetic composition is relatively similar.

### *Patterns related to life stage*

We evaluated possible associations between life stage and antipredator response by creating and examining data tables that compared the number of katydids in each life stage (i.e., juvenile and adult) that exhibited each of the possible alternative behaviors when stimulated with air flow or touch.

### *Patterns related to sex*

We evaluated possible associations between sex and antipredator response by creating and examining data tables that compared the number of katydids, separated by sex, that exhibited each

of the possible alternative behaviors when stimulated with air flow or touch.

#### *Patterns related to color*

We evaluated possible associations between color and antipredator response by creating and examining data tables that compared the number of katydids of each coloration (i.e., green, brown, or mottled green and brown) that exhibited each of the possible alternative behaviors when stimulated with air flow or touch.

#### *Patterns related to morphology*

We measured each katydid based on ten continuous morphological factors: body length, body width, body height, head length, mandible length, front leg length, middle leg length, hind leg length, left antenna length, and right antenna length. We recorded body length as the distance between the ends of the thorax and abdomen, body width as the largest lateral distance of the abdomen, and body height as the longest distance between the posterior and anterior of the body. We then performed a Principal Components Analysis (JMP13) to reduce the dimensionality of traits measured.

We evaluated possible associations between antenna length and antipredator response by creating and examining data tables that compared the number of katydids, organized by whether their longer antenna was shorter or longer than the mean longer antenna length, that exhibited each of the possible alternative behaviors when stimulated with air flow or touch.

We evaluated possible associations between body size and antipredator response by creating and examining data tables that compared the number of katydids, organized by whether they had a PC-1 score above or below the mean, that exhibited each of the possible alternative behaviors when stimulated with air flow or touch. We similarly evaluated possible associations between body shape and antipredator response by organizing katydids based on whether they had a PC-2 score above or below the mean.

## RESULTS

We collected 17 katydids belonging to the Pseudophyllinae (N=11), Agraeciinae (N=3), and Conocephalinae (N=3) subfamilies. Our sample included seven juvenile and ten adult katydids. There were eight females and nine males. Their colors could be classified as: green (N=5), brown (N=6), or mottled (N=6). Behavioral responses to stimuli were almost always the same for any individual katydid but varied among katydids (Table 1).

Table 1. The sequence of responses displayed by 17 katydids when exposed to (1) air flow and (2) tactile stimuli (touch). Each individual was tested three times with each stimulus. In almost all cases (all but 6%) each katydid exhibited the same response to each stimulus all three times. In the other cases, the table reports the modal response.

Katydid number	Stimulus	Response sequence					
		Initial response			Secondary response		
		Action	Antennae movement	Antennae directionality	Action	Antennae movement	Antennae directionality
1	air flow	freeze	fast	random	freeze	fast	random
	touch	freeze	slow	random	freeze	none	N/A
2	air flow	flinch	slow	at stimulus	freeze	none	N/A
	touch	flinch	slow	at stimulus	freeze	none	N/A
3	air flow	flinch	fast	random	freeze	none	N/A
	touch	flinch	fast	random	freeze	none	N/A
4	touch	flinch	slow	at stimulus	freeze	slow	at stimulus
5	touch	flinch	slow	random	walk	slow	at stimulus
6	air flow	jump	fast	random	walk	fast	random
	touch	flinch	fast	random	freeze	none	N/A
7	air flow	flinch	none	N/A	freeze	none	N/A
	touch	freeze	none	N/A	freeze	none	N/A
8	touch	flinch	slow	at stimulus	freeze	slow	random
9	air flow	flinch	slow	random	walk	slow	random
	touch	flinch	none	N/A	freeze	none	N/A
10	air flow	walk	fast	random	freeze	none	N/A
	touch	walk	fast	at stimulus	freeze	fast	random
11	air flow	flinch	fast	random	freeze	none	N/A
	touch	flinch	none	N/A	freeze	none	N/A
12	air flow	jump	none	N/A	freeze	none	N/A
	touch	jump	none	N/A	freeze	none	N/A
13	air flow	freeze	none	N/A	walk	slow	random
	tactile	flinch	fast	random	walk	fast	random
14	air flow	flinch	slow	at stimulus	freeze	none	N/A
	touch	jump	none	N/A	freeze	none	N/A
15	air flow	flinch	none	N/A	freeze	none	N/A
	touch	flinch	none	N/A	freeze	none	N/A
16	air flow	jump	fast	random	freeze	none	N/A
	touch	jump	fast	random	freeze	none	N/A
17	air flow	freeze	slow	random	walk	slow	random
	touch	flinch	slow	random	freeze	slow	random

*Patterns related to stimulus type*

Katydid sometimes directed their antennae towards tactile stimuli but never towards air flow stimuli during their secondary responses (Table 2).

Table 2. Katydid responses to air flow and a tactile stimulus. Values indicate number of katydid individuals. Blanks represent zeros.

Stimulus	Secondary antennae directionality	
	Random	At stimulus
Air flow	5	
Touch	4	2
Sum	9	2

*Patterns related to phylogeny*

As initial responses to either stimuli, Pseudophyllinae never walked, Agraeciinae always flinched, and Conocephalinae always walked or jumped (Tables 3a and 3b). Agraeciinae and Conocephalinae always froze and did not move their antennae as a secondary response to both stimuli (Tables 3a and 3b). Behavioral responses were more similar within subfamilies than any morphological trait; the only consistent trait within subfamilies was that all Conocephalinae were green (Supplementary table 1).

The two *Cocconotus wheeleri* were similar in their responses to stimuli. Both exhibited the same initial response (flinching), initial antennal movement (slow), secondary antennal movement (slow), and secondary antennal directionality (directed at stimulus) (Table 1 and Supplementary table 1). They differed only in initial antennal directionality and secondary actions (Table 1 and Supplementary table 1). The only other notable difference between these individuals was their sex (Supplementary table 1). Similarly, the two *Neoconocephalus affinis* individually exhibited the same behavioral sequence for both stimuli and differed from each other only in their initial antennal movement (no movement and fast movement) (Tables 1 and Supplementary table 1).

*Patterns related to life stage*

We found no difference in behavioral responses between juvenile and adult katydids.

Table 3a. Responses of katydid subfamilies to air flow. Values indicate number of katydid individuals. Blanks represent zeros.

Subfamily	Initial action				Secondary action		Secondary antennae movement	
	Freeze	Flinch	Walk	Jump	Freeze	Walk	No	Yes
Pseudo.	3	5		1	5	4	4	5
Agrae.		2			2		2	
Cono.			1	2	3		3	
Sum	3	7	1	3	10	4	9	5

Table 3b. Responses of katydid subfamilies to a tactile stimulus. Values indicate number of katydid individuals. Blanks represent zeros.

Subfamily	Initial action				Secondary action		Secondary antennae movement	
	Freeze	Flinch	Walk	Jump	Freeze	Walk	No	Yes
Pseudo.	2	8		1	9	2	7	4
Agrae.		3			3		2	1
Cono.			1	2	3		2	1
Sum	2	11	1	3	15	2	11	6

*Patterns related to sex*

Females never jumped as an initial response to stimuli, and males never walked as an initial response to stimuli (Tables 4a and 4b). Our samples of the two sexes were quite heterogenous with respect to subfamily, life stage, color, and morphology. (Supplementary table 1).

Table 4a. Responses of katydid sexes to air flow. Values indicate number of katydid individuals. Blanks represent zeros.

Sex	Initial action			
	Freeze	Flinch	Walk	Jump
Male	1	3		3
Female	2	4	1	
Sum	3	7	1	3

Table 4b. Responses of katydid sexes to a tactile stimulus. Values indicate number of katydid individuals. Blanks represent zeros.

Sex	Initial action			
	Freeze	Flinch	Walk	Jump
Male	1	5		3
Female	1	6	1	
Sum	2	11	1	3

*Patterns related to color*

Green katydids never froze initially and always froze secondarily. They never moved their antennae secondarily when stimulated with air flow (Tables 5a and 5b). Brown and mottled katydids never walked as an initial response (Tables 5a and 5b). The green katydid did not move to a green background when given the opportunity, nor did a brown katydid move to a brown background. Katydids of different colors came from different subfamilies and were variable in life stage, sex, and morphology. (Supplementary table 1).

Table 5a. Responses of katydids with different coloration to air flow. Values indicate number of katydid individuals. Blanks represent zeros.

Color	Initial action				Secondary action		Secondary antennae movement	
	Freeze	Flinch	Walk	Jump	Freeze	Walk	No	Yes
Green		1	1	2	4		4	
Brown	1	4		1	3	3	3	3
Mottled	2	2			3	1	2	2
Sum	3	7	1	3	10	4	9	5

Table 5b. Responses of katydids with different coloration to a tactile stimulus. Values indicate number of katydid individuals. Blanks represent zeros.

Color	Initial action				Secondary action		Secondary antennae movement	
	Freeze	Flinch	Walk	Jump	Freeze	Walk	No	Yes
Green		2	1	2	5		3	2
Brown	1	5			5	1	5	1
Mottled	1	4		1	5	1	3	3
Sum	2	11	1	3	15	2	11	6

*Patterns related to morphology*

Individuals with long antennae did not jump or walk for either stimulus (Tables 6a and 6b).

Katydids with short or long antennae came from different subfamilies and were of different life stages, sexes, and colors (Supplementary table 1).

Table 6a. Responses of katydids with short ( $50 \pm 5$  mm) and long ( $134 \pm 10$  mm) antennae to air flow. Values indicate number of katydid individuals. Blanks represent zeros.

Antennae	Initial action			
	Freeze	Flinch	Walk	Jump
Short	1	4	1	3
Long	2	3		
Sum	3	7	1	3

Table 6b. Responses of katydids with short ( $50 \pm 5$  mm) and long ( $134 \pm 10$  mm) antennae to a tactile stimulus. Values indicate number of katydid individuals. Blanks represent zeros.

Antennae	Initial action			
	Freeze	Flinch	Walk	Jump
Short		6	1	3
Long	2	5		
Sum	2	11	1	3

PC-1 accounted for 56% of the variance within the traits and was interpretable as body size (positive loadings of all factors; Table 7). PC-2 accounted for an additional 18% of the variance and reflected body shape. The lengths of legs and antennae loaded negatively, while head and mandible size loaded positively (Table 7). Large katydids (PC-1 score > mean) always flinched or froze as an initial response (Tables 8a and 8b). Large and small katydids came from different subfamilies and were variable in life stage, sex, and color. There were no obvious behavioral correlates with PC-2.

Table 7. Principle components analysis loadings scores for ten katydid morphological traits. (N=17 katydids)

Loading	PC-1	PC-2
Body length	0.35	0.08
Body width	0.34	0.07
Body height	0.37	0.16
Head	0.23	0.50
Mandible	0.24	0.52
Front leg	0.30	-0.43
Middle leg	0.36	-0.13
Hind leg	0.36	0.04
Left antennae	0.29	-0.47
Right antennae	0.29	-0.17
Variance Explained (%)	56	18

Table 8a. Responses of small and large katydids (below vs. above average scores for PC-1) to air flow. Values indicate number of katydid individuals. Blanks represent zeros.

PC-1	Initial action			
	Freeze	Flinch	Walk	Jump
Low	1	5	1	3
High	2	2		
Sum	3	7	1	3

Table 8b. Responses of small and large katydids (below vs. above average scores for PC-1) to a tactile stimulus. Values indicate number of katydid individuals. Blanks represent zeros.

PC-1	Initial action			
	Freeze	Flinch	Walk	Jump
Low		7	1	3
High	2	4		
Sum	2	11	1	3

## DISCUSSION

Individual katydids displayed the same behavioral sequence for a given stimulus across multiple trials but exhibited a wide variety of patterns based on stimulus type, phylogeny, sex, and morphology. These results suggest that antipredator strategies do not occur randomly but rather have likely evolved to best suit each organism, creating behavioral syndromes rooted in predator type and natural history.

The observed behavioral difference in response to air flow and touch can likely be explained by katydids' differential ability to locate predators that generate these signals: although katydids can detect the initial direction of air flow, secondary attempts to locate the source would



likely be futile given that flying, jumping, or otherwise fast-moving predators could have moved anywhere in the elapsed time. Katydids can similarly detect the initial direction of a predator that touches them, yet they can also locate predators by secondary antennal movement, as predators might not move after initial contact and might release informative pheromones that antennae can detect (Schneider 1964).

Phylogenetic patterns in antipredator responses are likely attributable to similar subfamily genetic compositions, which lead to conserved behaviors. The Agraeciinae and Conocephalinae subfamilies share more evolutionary history than any other pairing; this likeness could explain why members of these phylogenetic groups displayed the same secondary responses while members of the Pseudophyllinae subfamily did not (Naskrecki 2000). It remains unclear why the observed responses evolved within each of the three subfamilies, as the Conocephalinae response cannot be explained by their shared coloration, and no other observed trait was constant within or across subfamilies. Future studies could investigate other possible traits that generate these behavioral syndromes. Contrarily, specific traits might not cause phylogeny-dependent behavioral sequences. Perhaps these responses arose due to the environments that the subfamilies occupy, or perhaps it is evolutionarily beneficial for subfamilies to evolve unique antipredator responses to avoid predictability.

Juveniles and adults might not differ in antipredator responses due to the benefit of developing these behaviors as early as possible. Juvenile survivorship might be too low if these individuals have inadequate abilities to avoid predation.

Sex differences such as parental care, mating behaviors, and hormone levels might explain the observed differences in male and female responses (Ryan 1998). For instance, perhaps females do not jump in response to a potential predator because they cannot afford to abandon their eggs or young (Lang et al. 2007). Male katydids are not known to display site fidelity and thus might always jump if they are

to change location to evade a predator rather than walk, as jumping more drastically changes an individual's location (Lang et al. 2007). Katydids with longer antennae might not jump or walk to avoid predators because they can better detect stimuli at a distance and thus have more time to react when a stimulus poses a legitimate threat. Furthermore, long antennae might make an individual too conspicuous with movement, whereas short antennae are less visibly detectable.

Crypsis might help explain associations between katydid coloration and antipredator responses. Green katydids are known to occupy green environments such as leaves; therefore, they might always initially move in order to best position themselves for disguise and subsequently always freeze their bodies so as to camouflage as effectively as possible (Gwynne 2001). They might continue to move their antennae while frozen for tactile stimuli, as a relatively high probability of detecting the predator outweighs the risk of slight movement. On the other hand, green katydids might freeze their antennae as well as their bodies when stimulated with air flow, as the relatively low probability of detecting a flying or otherwise quickly-moving predator does not outweigh the risk of movement. Brown and mottled katydids might have exhibited the same patterns in antipredator response because they occupy similar environments such as sticks, tree bark, and soil (Gwynne 2001). These individuals might not walk as an initial response to predator stimuli because this motion is more detectable on brown substrates due to the fact that they vary more in coloration within a walking distance than a leaf. Although we found that green and brown katydids do not respectively choose green and brown environments, the experiment was conducted in a highly artificial setting. Future studies should investigate katydid habitat choice in field.

Given that PC-1 is correlated with overall larger body size and body parts, katydids that had high PC-1 scores might not have walked or jumped as an initial action due to the high energetic costs of these movements. However, a more probable explanation emerges from the fact that the observed pattern based on antenna

length is identical to that based on PC-1. This result is noteworthy because a single variable can account for all patterns that emerge by combining ten. Perhaps antennae length is the morphological trait that most greatly impacts katydid antipredator responses because it is the only feature that confers differential predator detection abilities as a factor of size. PC-2 likely did not dictate any patterns in the observed behaviors because it accounted for only a minor amount of variation in katydid morphology. Two notable complications arose in this study. Due to the small quantity of katydids tested, behavior presence and thus absence was often contingent on one individual; therefore, it is probable that there a katydid might have fallen into an otherwise absent category (e.g., a female who jumped) but was simply not collected. Additionally, we performed experiments on a white sheet, which is both a color and substrate that katydids do not naturally occupy. Future replications could be conducted in the field to investigate more organic responses.

Successful antipredator strategies greatly improve organism fitness, yet their patterns and causes are often little understood. This study provides insight into the elaborate and variable nature by which prey behave in response to various stimuli, supporting the notion that even subtle antipredator strategy differences are not random but rather have likely evolved to suit their organisms in regard to predator type and natural history. It is evident that there is not always one optimal response to predators but rather an exceedingly wide variety of behavioral syndromes that confer unique advantages based on sequential order.

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#### AUTHOR CONTRIBUTIONS

Both authors contributed equally.

#### LITERATURE CITED

- Dominici, P. 2008. Cockroaches keep predators guessing by using preferred escape trajectories. *Current Biology* 22:1792-1796.
- Fain, G. 2003. Sensory transduction. Sunderland, Massachusetts: Sinauer Associates, Inc. Publishers.
- Gwynne, D. T. 2001. Katydid and bush crickets: reproductive behavior and evolution of the Tettigoniidae. Ithaca, New York: Cornell University Press.
- Lang, A. B. & Heinrich, R. 2007. Roost site selection and site fidelity in the Neotropical katydid *Docidocercus gigliotosi* (Tettigoniidae). *Biotropica* 40:183-189.
- Lardner, B. 2000. Morphological and life history responses to predators in larvae of seven anurans. *Synthesizing Ecology* 88:169-180.
- Naskrecki, P. 2000. Katydid of Costa Rica. Philadelphia, Pennsylvania: The Orthopterists' Society.
- Ryan, M. J. 1998. Sexual selection, receiver biases, and the evolution of sex differences. *Science* 281:1999-2003.
- Schneider, D. 1964. Insect antennae. *Annual Review of Entomology* 9: 103-122.
- Sih, A. et al. 2004. Behavioral syndromes: an integrative overview. *The Quarterly Review of Biology* 79: 241-277.
- Tau, G. Z., & Peterson, B.S. 2010. Normal development of brain circuits. *Neuropsychopharmacology* 35: 147-16.

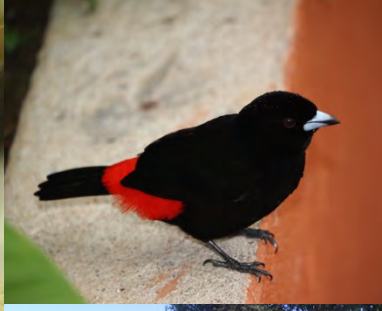
Supplementary Table 1. Phylogenetic and morphological information of the katydids examined. For subfamilies, "P" denotes Pseudophyllinae, "A" denotes Agracilinae, and "C" denotes Conocephallinae. For life stage, "J" denotes juvenile and "A" denotes adult. If not otherwise indicated, trait lengths were measured. For sex, "F" denotes female and "M" denotes male. For color, "G" denotes green, "B" denotes brown, and "M" denotes mottled brown and green. All measurements are in millimeters.

Katydid number	Species	Subfamily	Life stage	Sex	Color	Body length	Body width	Body height	Head	Mandible	Front leg	Middle leg	Hind leg	Left antennae	Right antennae
1	<i>Coccothotus degreii</i>	P	A	F	M	37.5	9.7	12.0	9.0	3.8	27.9	32.5	55.7	112.0	96.0
2	<i>Subria</i> spp.	A	A	F	B	13.2	6.0	9.1	7.9	3.5	18.7	20.7	49.6	51.2	55.9
3	<i>Scopiorhinus</i> spp.	P	J	F	G	22.0	4.6	5.2	6.3	1.2	25.9	20.5	43.1	140.8	34.9
4	<i>Coccothotus wheeleri</i>	P	A	F	M	27.5	7.9	8.1	8.0	4.9	22.1	26.9	50.2	115.4	166.5
5	<i>Coccothotus wheeleri</i>	P	A	M	M	28.2	8.1	8.3	9.0	3.9	26.7	27.1	48.1	125.8	81.8
6	Unknown A	P	J	M	B	22.8	6.8	6.7	6.2	2.8	7.9	3.9	38.6	29.6	60.2
7	<i>Docidocerus gigliotosi</i>	P	A	M	B	24.3	6.6	8.4	7.3	2.7	25.9	26.8	48.2	138.5	123.3
8	Unknown B	A	J	M	G	9.0	3.0	2.7	4.5	2.7	23.5	18.3	36.6	51.2	42.2
9	Unknown C	P	J	M	B	15.5	6.1	3.9	4.5	1.6	19.2	13.8	37.0	32.3	12.5
10	<i>Erioloides</i> spp.	C	A	F	G	17.2	5.7	7.1	7.4	3.2	12.6	14.8	29.0	36.8	55.4
11	<i>Subria</i> spp.	A	A	F	B	14.8	5.8	7.2	8.2	3.5	18.0	18.6	49.9	53.0	38.9
12	<i>Neococephalus affinis</i>	C	A	M	G	18.4	5.4	7.1	9.9	3.9	16.5	18.6	44.6	44.6	35.2
13	Unknown D	P	J	F	B	22.0	6.2	6.4	7.5		18.1	19.5	36.4	12.4	25.2
14	Unknown E	P	J	M	M	23.6	6.2	4.9	6.5	3.6	18.3	15.9	35.1	77.3	77.1
15	Unknown F	P	J	F	M	33.4	9.8	13.4	9.5	5.1	29.2	26.9	56.4	95.1	93.8
16	<i>Neococephalus affinis</i>	C	A	M	G	22.2	4.1	5.3	11.7	4.4	14.5	19.4	44.8	35.8	47.6
17	Unknown G	P	A	M	M	20.2	7.1	8.9	7.4	2.6	25.2	20.5	46.1	132.1	159.9











RAINING STICKS AND STONES: FOOD RECOGNITION IN A TROPICAL FRUGIVOROUS FISH  
*BRYCON GUATEMALENSIS*

ALEXANDER W. COTNOIR, BALTHASAR L. VON HOYNINGEN HUENE, CLAYTON E. JACQUES, AND MARY M. PEDICINI

Teaching Assistants: Michael B. Brown and Deb Goedert  
Faculty Editor: Matthew P. Ayres

**Abstract:** Organisms face limits to optimizing their foraging efficiency, such as sensory perception of food items. The neotropical freshwater fish *Brycon guatemalensis* feeds primarily on allochthonous stream inputs, particularly fruit, which it must distinguish from inedible debris. We tested the hypothesis that *B. guatemalensis* uses object characteristics to differentiate among potential food items. We introduced edible and inedible objects into streams and quantified the reactions of fish using an ethogram. *B. guatemalensis* displayed stronger foraging activity around the food item (pineapple) than three inedible items (wood, stones, and nut shells). Furthermore, the fish habituated to the repeated input of inedible items, losing interest at equivalent rates across all types of items. *B. guatemalensis* has demonstrated food recognition and habituation abilities specialized for its complex foraging environment.

**Keywords:** *Brycon guatemalensis*, food recognition, foraging behavior, habituation

INTRODUCTION

Optimal foraging theory describes strategies by which organisms can maximize foraging efficiency. The extent to which an organism can optimize foraging behavior is limited by its ability to perceive the environment and make appropriate decisions. For instance, organisms face the challenge of identifying food items. This can be especially challenging for opportunistic foragers, which must distinguish between non-food items and a variety of potential food items. Because the pursuit of potential food involves costs such as energy expenditure and increased exposure to predators, natural selection must favor increased ability to make such distinctions. On the other hand, opportunistic foragers may rely on experimenting with new potential food sources, unlike more specialized foragers. Therefore, organisms that utilize a diverse number of food resources would improve foraging efficiency by more accurately identifying genuine food items and minimizing pursuit of inedible items, while maintaining some degree of flexibility in response to new items they encounter. That is, individuals may experiment with an item at first encounter, but learn and/or habituate from this initial experience.

These issues are relevant to the foraging behavior of *Brycon guatemalensis*, a frugivorous

neotropical freshwater fish. *B. guatemalensis* feed on the fruit and leaves of *Ficus* trees.

Opportunistically, these fish also feed on drifting materials, which it must identify as food amid a frequent barrage of inedible debris such as sticks and detritus (Horn 1996). Under some circumstances, *B. guatemalensis* habituate to consistent inedible inputs (Engler et al. 2011) and appear to use visual cues from inputs and movements of conspecifics to locate potential food items.

We investigated 1) the ability of *B. guatemalensis* to distinguish among food and non-food inputs and 2) if they habituate equally to different inedible stream inputs. *B. guatemalensis* may react equally to any stream surface disturbance of an appropriate magnitude, irrespective of object characteristics.

Alternatively, *B. guatemalensis* may be able to distinguish among inputs based on the object's buoyancy. In this case, the fish would have difficulty distinguishing inedible items with similar buoyancy properties as actual food items. Given the dependency of *B. guatemalensis* on bobbing fruits (primarily figs), it could be that they would more intensely pursue bobbing non-food items (empty nut shells) than sinking (pebbles) or floating (wood) non-food items. If so, fish should initiate foraging activity around inedible items that emulate the buoyancy of the



fish's natural food items while ignoring equally inedible food items with different buoyancy. By the same logic, habituation may occur more quickly to easily distinguished inedible items (pebbles or wood) than to the food-emulating items (nut shells).

## METHODS

### *Site selection*

We conducted our experiments at 8 locations near 4 trail crossings on the El Santito and El Pantano rivers at La Selva Biological Station in Costa Rica. At each crossing, we attempted to conduct trials at the crossing itself, and at sites at least 20 meters up- and downstream. We identified stream patches with good visibility at each site. Because topography and vegetation limited the accessibility of the up- and downstream sites, reducing our total number of sites was restricted to 8. Our observations indicate that we were studying different fish at each of the 8 sites.

### *Experimental trials*

Before beginning our experimental trials, we attracted fish by throwing objects (pebbles, wood fragments, occasionally pieces of cookie) into the stream center. Each trial consisted of four rounds, spaced apart by 5-minute cool-down periods. In the first round, we introduced chunks of pineapple (2 cm cubes) into the same patch of river, throwing 10 chunks at 5-second intervals. This allowed us to collect baseline fish responses to food items with no effect of habituation.

In the second and third rounds, we repeated the procedure with 30 inedible objects. In one of these rounds, we used sections of wood approximately 2-3 cm<sup>3</sup> in volume, while in the other, we used small pebbles of approximately the same volume. We randomized the item between second and third rounds so as to avoid confounding effects of order. These objects allowed us to test fish responses to inedible objects that differ in physical properties (specifically buoyancy), as well as to reveal any patterns of habituation.

In the fourth and final round, we introduced 30 half-nut shells at 5-second intervals in the manner of the prior rounds. Once all 30 shells

had been introduced, we waited 5 seconds and then switched to pineapple chunks, introducing 10 at 5-second intervals. By transitioning from inedible to edible items, we intended to reveal any effects of short-term habituation on food recognition.

We videotaped each round of each trial for later analysis. Depending on the characteristics of the site, we used either above- or below-surface cameras, or both when possible. We also recorded written notes as each trial progressed.

### *Video analysis*

We examined video recordings of each trial in order to observe and measure individual fish responses over time. For each dropped item, we quantified fish foraging responses using an ethogram. We used the ethogram of Engler et al. (2011) from their study of *B. guatemalensis* at La Selva. Scores were assigned accordingly: 0 = no response to item, 0.25 = slight movement towards item, 0.5 = minor effort to investigate item, 1 = active investigation, 2 = effort to eat item, 3 = aggressive effort to eat item. This ethogram produced a score for each visible fish at each dropped item. The higher the score, the greater the strength of foraging response the individual exhibited to the potential food item.

### *Statistical analysis*

We calculated the average strength of response for all fish at each drop of a potential food item by summing the ethogram scores for all individuals and dividing by the size of the group. To test the abilities of *B. guatemalensis* to distinguish by potential food item, we examined the average strength of response by input type for the first 10 drops using a mixed model ANOVA, with input type as a fixed effect and site as a random effect. To test the habituation capacity of the fish to inedible items, we investigated the strength of response by the drop sequence of the input with a linear mixed model, with site as a random effect. We accounted for input type, the drop sequence, and their interaction within the model.

## RESULTS

We recorded fish responses to 870 drops of potential food items within 4 rounds at 8 stream

sites. We usually observed fish in groups of 4 to 10 individuals (mean  $\pm$  SD =  $3.9 \pm 2.7$ ). We observed zero fish during many of our drops involving inedible food items. The group sizes sometimes changed after we began manipulations, typically increasing in size.

The strength of foraging responses varied with the type of input (Figure 1;  $F_{3,309} = 46.65$ ,  $P < 0.0001$ ); the fish reacted most strongly to the input of actual food (pineapple) than to the inedible items (pebble, wood, nut shell), regardless of item similarity to food. In our analysis, 14% of the total random variance was attributable to differences among sites.

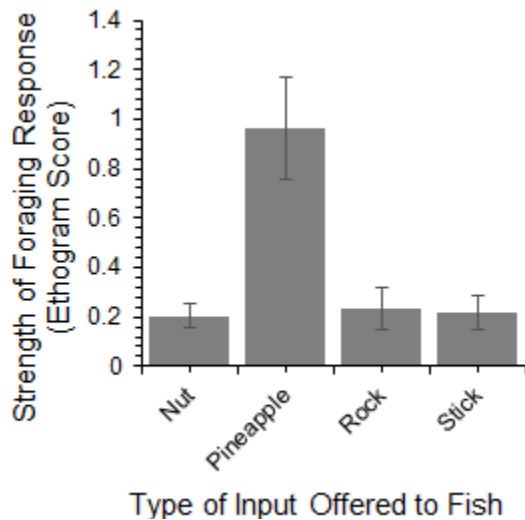


Figure 1. Strength of foraging response (mean  $\pm$  SE) for fish at eight locations that were offered similarly sized objects of four types.

*B. guatemalensis* habituated to offerings of non-food items ( $F_{1,707} = 73.87$ ,  $P < 0.0001$ ) but foraged even after 30 consecutive offerings of one (Figure 2). We found no interaction between input and drops ( $F_{2,707} = 0.87$ ,  $P = 0.42$ ), indicating that the fish do not habituate differently to the range of inedible items we provided.

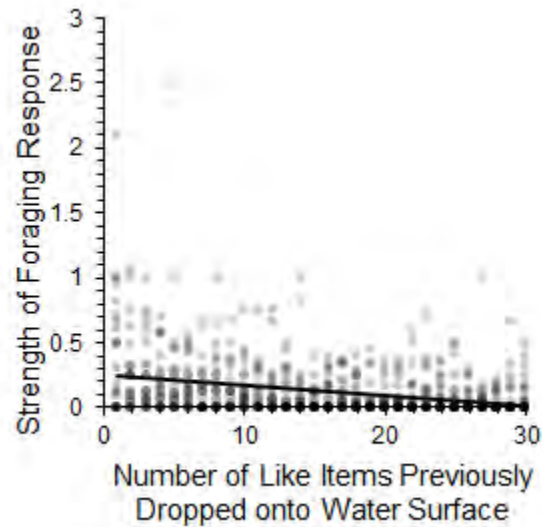


Figure 2. Strength of foraging response decreased as the trial continued. Line of fit is a least squares regression line. Darkness of points indicates number of observations.

## DISCUSSION

*B. guatemalensis* clearly distinguished among allochthonous inputs, with stronger foraging for edible inputs. However, fish did not distinguish inedible inputs, displaying equal levels of interest despite the inputs' different buoyancy properties. This finding contradicts our hypothesis that *B. guatemalensis* uses cues related to the buoyancy of an object to identify food items. Although fish demonstrate habituation for repeated inedible inputs, we found no support for the hypothesis that these fish habituate less to inedible stream inputs with similar buoyancy as actual food items compared to inedible floating or sinking objects. Collectively, our findings reject the null hypothesis that fish react equally to surface disturbance of a certain magnitude.

The ability of *B. guatemalensis* to adjust foraging behavior based on perceived patterns of inedible food inputs provides an adaptive advantage. Abruptly recognizing an inedible item after few encounters allows the fish to minimize wasteful energy expenditure and exposure to potential predators.

How *B. guatemalensis* forages in a group context and distinguishes food from non-food remains a topic of interest. If a fish sees a conspecific eat an item, the desirability may

increase. On many occasions, we observed rapid congregations of fish forming after a single individual approached and consumed an item, indicating that individuals may monitor the behavior of surrounding conspecifics to detect food.

During the trials, we noticed that the fish switched from habituation to nut shells to a feeding frenzy after only several pineapple chunks entered the stream. Therefore, *B. guatemalensis* may utilize more advanced visual or olfactory cues to distinguish food from non-food items than those investigated by this study. Future studies could investigate this possibility.

*B. guatemalensis* live in an environment where food is both temporally and spatially unpredictable, interspecific competition for food resources is high, and bombardment by inedible items into the water is a near constant occurrence. In such a challenging environment, accurately and quickly distinguishing between food and non-food items is important if organisms are to minimize unnecessary energy expenditure and effectively increase their fitness. Natural selection favors individuals within such an environment that possess a combination of attuned sensory perceptions and

adaptability to ensure efficient resource acquisition within a complex foraging scene. Our findings provide interesting insights into how species that employ sit-and-wait strategies exploit temporally unpredictable food resources.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

- Engler, I. D., Wang, F. M., and R. M. Niemc. 2011. Grouping behavior of the frugivorous freshwater tropical fish *Brycon guatemalensis*. *Dartmouth Studies in Tropical Ecology* 2011: 90-94.
- Horn, M. H. 1996. Evidence for dispersal of fig seeds by the fruit-eating characid fish *Brycon guatemalensis* in a Costa Rican tropical rainforest. *Oecologia* 109: 259-264.

## THE RESPONSE OF EUGLOSSINE BEES TO RARITY IN NOVEL SCENTS

ALEC B. COBBAN, ALEXANDRA M. STENDAHL, AND PAUL J. VICKERS

Teaching Assistants: Michael Butler Brown and Debora Goedert

Faculty Editor: Matt Ayres

*Abstract:* The relationship between euglossine bees (orchid bees) and orchids provides an apparent example of asynchronous diversification in which orchids may have speciated by taking advantage of a predisposed behavior of male orchid bees to seek out and collect novel scents for use in sexual selection. The increased pollination for orchids producing new scents could rapidly lead to reproductive isolation. We investigated orchid bees' innate scent preferences and selection for locally rare scents compared to locally common novel scents. In 30 hours of observation at lures baited with a variety of scented essential oils, there was no difference in bee abundance between primary forests and forest edge habitat. Additionally, we observed a trend towards selection of a specific scent and preferences for locally rare scents compared to locally abundant scents. Our results were consistent with the hypothesis that the scent collection behavior of orchid bees may be responsible for the asynchronous diversification seen in orchids.

*Key words:* coevolution, orchid bees, rarity, scent

### INTRODUCTION

The Neotropics are globally notable for high biodiversity. One hypothesis for this high level of diversity posits that biotic interactions will facilitate finer niche space through processes such as mutualisms (Ramirez et al. 2011). The plant-pollinator relationship is often highlighted as an example of this type of coevolution; however, recent work has indicated asymmetrical diversification may better explain these patterns (Bascompte and Jordano 2007). The context for our studies focused on the relationships between euglossine bees (orchid bee) and orchids, which have historically been viewed as a classic example of coevolution. There are over 200 species of orchid bees in the Neotropics. They are thought to be essential for pollination in 600 species of orchids, which is about 10 percent of the diversity of Neotropical Orchidaceae (Ramirez et al. 2011).

Orchid bees (Figure 1) are relatively common and taxonomically diverse. Both sexes are solitary and are capable of flying over 10 kilometers a day foraging for food (Roubik 2004). Additionally, males collect and store scent volatiles which are believed to be used in sexual selection (Roubik 2004). Recent work by Ramirez et al. (2011) indicates that the diversification of orchids was later than the diversification of orchid bees, contrary to the

earlier hypothesis of synchronous radiation. A possible mechanism for this diversification is the exploitation of a pre-existing tendencies in orchid bees to preferentially collect rare scents, which could have permitted rapid reproductive isolation in orchids that evolved novel scents. Orchid bees have been reported to prefer some scents over others but also to favor scent diversity per se (Eltz et al. 1999; Eltz et al. 2005). Different forest types such as forest edge, primary forest, and secondary forest can sometimes, but not always, influence the community composition of orchid bees, which could have further effects on scent preferences if bee species vary in their preferences (Roubik and Hanson 2004, Nemesia and Silveira 2006, Billipp et al. 2013).



Figure 1: Specimen of *Euglossa*

We tested whether orchid bees prefer scents that are locally rare. Our study design allowed for the possibility that orchid bees have relatively fixed preference for specific scents that are independently of local abundance of the scent. We presented the same arrays of scent choices in primary forest and forest-edge habitats, which also permitted comparisons of bee abundance by habitat.

## METHODS

We conducted this study at La Selva Biological Station in Costa Rica. To determine whether orchid bees respond to rarities of scents at a fine temporal and spatial scale, we created an experimental array where scents were locally abundant or locally rare. Scent combinations were made by adding 10 drops of vanilla or wintergreen essential oil to 20 ml of ethanol and dipping kimwipes in the solution. Kimwipes were clothespinned directly onto leaves to act as orchid bee lures. Both scents were chosen because of their association with orchids: wintergreen contains primarily methyl salicylate, which occurs in 4 percent of orchids and attracts 20 percent of orchid bee species (Dodson 1969). Vanilla extract comes from the seed pods of vanilla orchids (*Vanilla* spp.), which are native to central America. Although the compound, vanillin, is not produced by an orchid obligatorily pollinated by orchid bees, it resembles the fragrance of the obligatory pollinated orchid *Stanhlopha ocellata* (Dodson 1969).

For each array we placed five lures approximately one meter apart; we qualified 4 lures of the same scent as locally abundant (Figure 2, wintergreen), and 1 lure of a different scent as locally rare (Figure 2, vanilla). We deployed each array with a pair array of opposite configuration approximately 100 m apart. We placed an equal number of array pairs in primary forest and forest-edge environments.

We deployed scent lures an hour before beginning observation. We conducted all observations between 9:00 and 15:00 (30 total hours of observation). Each observational period involved watching one array for 30 minutes. We randomized order between days to account for temporal difference. For every orchid bee

observed, we recorded which scent it selected, and then attempted to catch it. We described bees that we captured described and identified to genus using the key in Roubik and Hansen (2004).



Figure 2. Experimental setup in which vanilla was locally rare (forest edge habitat).

## Statistical Analysis

We used chi-square statistics to test for differences in orchid bee visits between habitats, preference for scent type, and preference for rare or common scents.

## RESULTS

We observed a total of 43 bees and collected 9 to identify (Supplementary Table 2). We found the same 3 genera of euglossini bees in both edge and forest habitats (*Eulaema*, *Euglossa*, *Exaerete*). Low sample sizes limited inferences regarding species composition. We recorded only four bees on the second day of sampling, when it was raining, and only two bees on the third day, despite the weather being mostly sunny. Because of low sample size, bees observed on these days were not included in analysis.

Orchid bees showed no preference for primary forest or forest edge (chi-square = 0.33,  $p = 0.56$ ,  $df = 1$ ; Table 1). They tended to prefer wintergreen scent over vanilla, though the result was not significant (chi-square = 3.00,  $p = 0.08$ ,  $df = 1$ ; Table 2). Orchid bees showed a clear preference for rare scents over common scents (chi-square = 7.26,  $p = 0.007$ ,  $df = 1$ ; Table 3). Regardless of the scent that was rare, we observed nearly as many bees visiting the rare scent in an array, even though the rare scent was only one of the five lures within the array. Bee visits to all eight deployed arrays are in Supplementary Table 1.



Table 1: Orchid bee sightings at scent lures in forest vs. edge habitats. The predicted value is half of the total sightings because there was equal sampling intensity in the two habitats.

Location	Predicted	Actual
Forest	13.5	15
Edge	13.5	12

Table 2: Orchid bee sightings at vanilla scent lure vs. wintergreen scent lure. Predicted values were half of the total sightings because the number of lures and array types were equal for both scents.

Scent	Predicted	Actual
Vanilla	13.5	9
Wintergreen	13.5	18

Table 3: Orchid bee sightings at scents that were common vs. rare within arrays. Predicted values were 1:4 of total sightings because the arrays were 1:4 for rare vs. common scents.

Scent		
Abundance	Predicted	Actual
Rare	5.4	12
Common	21.6	15

Available information on taxonomy and morphology are in Supplementary Table 2.

## DISCUSSION

Orchid bees did not exhibit a preference for forest edge or primary forest for habitat types. Our results suggest that edge effects do not influence abundance of orchid bees. Orchid bees might occasionally benefit from forest edges because scent may be easier to detect at the edge, and bees might make excursions to the edge to collect different scents (Nemesio and Silveira 2006). Additionally, our results indicate that orchid bees prefer locally rare scents, and demonstrate preferences for some scent types. Scent preference is consistent with previous work, which found that orchid bees were more attracted to specific scents when presented with options (Billipp 2013). Orchid bee preference for rarity supports the hypothesis that this behavior leads to orchid speciation, which is consistent with the observed asynchronous diversification. The displayed preference for rarity among novel scents suggests that orchids which mutate to produce a new scent will have a

better chance of being visited and therefore pollinated.

This study examined how scent abundance in local environments influences orchid bee attraction. It is possible that the preference for rare scents in our study is related to “sensory adaptation,” a phenomenon from animal physiology in which chemoreceptors become less responsive to stimulation after a brief period of sustained stimulation. On a greater timescale, bees may use memory to discriminate among scents based on whether they have already been collected over the previous days. Eltz et al. (2005) showed that once orchid bees collect a scent, they are no longer attracted to that scent for several days, which implies capacity to remember scents that they have recently collected (Eltz et al. 2005).

Further studies could add to understanding of the scale of preferences for scents that are rare vs. common in the home range of individuals bees. Because both scents presented to the orchid bees in this study contained compounds that are found in orchids in Central America, both could be considered familiar to these species at least on an evolutionary scale. However, as both scents come from essential oils, they are highly purified and have additional compounds, so could simultaneously be considered novel. Other future directions include studying how different genera or species prefer different scents and habitats.

Apparently, both local abundance of scents and orchid bee scent preferences influence orchid bee behavior. Orchid bees prefer rare scents on a small spatial scale and have preferences for some scents over others. Because many orchids rely solely on orchid bees for pollination, orchid bee behavior and scent preference could be a major selection force and could have been influential in the evolution of orchids. Our study, though modest in scale, adds to the understanding of a classic model of coevolution in the plant-pollinator relationship. Further study of similar systems, especially comparative studies of temperate species, could further inform understanding of biotic interaction influencing patterns in biodiversity.

## ACKNOWLEDGEMENTS

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## AUTHOR CONTRIBUTIONS

All authors contributed equally.

## LITERATURE CITED

- Bascompte, B. and Jordano, P. 2007. Plant-animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics* 38:567-593.
- Billipp, T. E., C. P. Cowdery, E. H. Hull. 2013. Habitat selection in euglossine bees in Corcovado, Costa Rica. *Dartmouth Studies in Tropical Ecology* 2013, pp. 99-104.
- Dodson, C. H., Dressler, R. L., Hills, H. G., Adams, R. M., & Williams, N. H. 1969. Biologically active compounds in orchid fragrances. *Science*, 164(3885), 1243-1249.
- Eltz, T., Whitten, W. M., Roubik, D. W., and K. E. Linsenmair. 1999. Fragrance collection, storage, and accumulation by individual male orchid bees. *Journal of Chemical Ecology* 25: 157-176.
- Eltz, T., Roubik, D. W., and Lunau K. 2005. Experience-dependent choices ensure species-specific fragrance accumulation in male orchid bees. *Behavioral Ecology and Sociobiology* 59:149
- Nemesio, A. and F. A. Silveria. 2006. Edge effects on the orchid-bee fauna (Hymenoptera: Apidae) at a large remnant of atlantic rain forest in Southeastern Brazil. *Neotropical Entomology* 35(3): 313-323.
- Nemesio, A. and F. A. Silveria. 2007. Orchid bee fauna (Hymenoptera: Apidae: Euglossina) of Atlantic forest fragments inside an urban area in Southeastern Brazil. *Neotropical Entomology* 36(2): 186-191.
- Roubik, D. W. and P.E. Hanson. 2004. *Orchid bees of tropical America: Biology and field guide*. INBio. Santo Domingo de Heredia, Costa Rica.

## SUPPLEMENTARY TABLES

Supplementary Table 1: Occurrence of common and rare visits by orchid bees in 8 treatments

Location	Site	Treatment	Rare	Common
Edge	Classroom	Rare Vanilla	2	5
Edge	Classroom	Rare Wintergreen	4	1
Edge	Classroom2	Rare Vanilla	0	0
Edge	Classroom2	Rare Wintergreen	0	0
Forest	CES200	Rare Vanilla	1	4
Forest	CES300	Rare Wintergreen	0	2
Forest	CES500	Rare Vanilla	2	3
Forest	CES600	Rare Wintergreen	3	0

Supplementary Table 2: During our study we were able to capture and measure 9 orchid bees. For an addition 14 individuals, we were able to record their color, scent choice and tentatively identify them to genus or species.

Genus	Species	Tongue Length (mm)	Body Length (mm)	Color	Forest/Edge	Location	Treatment	Date	Time	Scent Choice
Euglossa	imperialis			Metallic Green	Edge	Behind Classroom	Menu(1)	2/8/2018	10:50	Wintergreen
Euglossa				Blue	Edge	Behind Classroom	Menu(1)	2/8/2018	11:00	Vanilla
Eulaema				Bumblebee	Forest	CES100	Menu(2)	2/9/2018	07:56	Jasmine
Eulaema				Bumblebee	Forest	CES200	VW(Vanilla Rare)	2/9/2018	11:05	Wintergreen
Euglossa		6.6	9.8	Green and Copper	Forest	CES300	VW(Wintergreen Rare)	2/9/2018	10:10	Vanilla
Euglossa		6.6	10.6	Blue	Forest	CES300	VW(Wintergreen Rare)	2/9/2018	10:26	Vanilla
Eulaema				Bumblebee	Forest	CES400	Menu(2)	2/9/2018	10:40	Wintergreen
<del>Euglossa</del>	samarangolina	13.8	25.1	Green and Giant	Forest	CES400	Menu(2)	2/9/2018	11:07	Wintergreen
Eulaema				Bumblebee	Forest	CES400	Menu(2)	2/9/2018	16:03	Wintergreen
Eulaema				Bumblebee	Forest	CES500	VW(Vanilla Rare)	2/9/2018	11:27	Vanilla
Euglossa		6	11.4	Blue	Forest	CES600	VW(Wintergreen Rare)	2/9/2018	12:15	Wintergreen
Euglossa		6.2	10.6	Blue	Edge	Classroom	Menu(2)	2/9/2018	11:00	Jasmine
Euglossa		6.5	14.1	Green with Copper back	Edge	Classroom	VW(Vanilla Rare)	2/9/2018	09:36	Wintergreen
Euglossa		15.7	15.2	Bright Green	Edge	Classroom	VW(Vanilla Rare)	2/9/2018	09:40	Wintergreen
Euglossa		6.4	10.3	Blue	Edge	Classroom	VW(Vanilla Rare)	2/9/2018	09:56	Wintergreen
Eulaema				Bumblebee	Edge	Classroom	VW(Wintergreen Rare)	2/9/2018	10:15	Wintergreen
Eulaema				Bumblebee	Edge	Classroom	VW(Wintergreen Rare)	2/9/2018	10:15	Wintergreen
Euglossa		6.3	10.7	Blue	Edge	Classroom	VW(Wintergreen Rare)	2/9/2018	10:17	Vanilla
Euglossa	imperialis			Metallic Green	Edge	Meadow	Menu(1)	2/8/2018	10:15	Wintergreen
Euglossa	imperialis			Metallic Green	Edge	Meadow	Menu(1)	2/8/2018	10:15	Wintergreen
Eufesia	palidia			Black and Copper	Edge	Outside classroom	Menu(1)	2/8/2018	08:00	Wintergreen
Euglossa				Blue/Green and Copper	Forest	Sura 150	Menu(1)	2/8/2018	10:20	Wintergreen
Eulaema				Bumblebee	Forest	CES500	JV(Vanilla rare)	2/10/2018	10:10	Jasmine

## HOW SPECIES DEPENDENCY ON A RESOURCE DRIVES COMMUNITY STRUCTURE: A PROPOSED STUDY OF OBLIGATE AND FACULTATIVE FOLLOWERS OF ARMY ANTS

GRACE S. CALLAHAN AND MAANAV JALAN

Teaching Assistants: Debora Goedert and Michael B. Brown  
Faculty Editor: Matthew P. Ayres

**Abstract:** In communities surrounding a shared resource, there are often species that are more dependent on that resource than others. These species will invest more energy to ensure that they benefit from these resources, and incur greater losses if outcompeted. This results in communities with structures that are dictated by these species. To test this, we propose an examination of groups of army ant following birds at La Selva Biological Station in Costa Rica. These bird groups are made up of both birds that rely solely on the insects disturbed by the ant swarms (obligate birds) and birds that opportunistically feed around these swarms but don't depend on them for survival (facultative birds). We hypothesize that emergent properties of these groups such as size, composition, and patterns of change are driven by behavior of obligate ant birds. Results of this study could provide us with a deeper understanding of how communities around a resource are formed and maintained. Understanding these dynamics will allow us to predict how communities may change under varying conditions.

**Key words:** army ant-following birds, community structure, competition, *Eciton burchellii*, obligate and facultative

### INTRODUCTION

High quality food resources are often concentrated, resulting in the concentration of resource consumers as well. Such guilds may be composed of species with varying levels of dependence on the resource, which may have consequences for the structure and dynamics of the guild. The situation becomes even more complex if the food resources are variable and unpredictable.

Neotropical bird groups that forage around army ant (*Eciton burchellii*) swarms provides a model system to study guild structures around unreliable resources. These birds follow ant swarms and feed on insects flushed out by the ants (Willis 1983, Brumfield et. al 2007, Chaves-Campos 2011). Among the bird species that can be found foraging in and around army ant swarms, there are varying degrees of dependency on the ants. Such bird species have frequently been classified as obligate or facultative followers of army ants (Garrigues and Dean 2014). Obligate species depend on insects flushed out by army ants as a major food source and do not occur in forests that lack army ants. On the other hand, facultative species opportunistically forage with army ants but can also be found in forests that lack such resources (Willis and Oniki 1978).

While army ant swarms can provide copious food when available, they are unreliable. Ant colonies do not forage every day (only 65-75% of days) and are nomadic, moving 100-200 meters each day and creating a bivouac (a nightly aggregation of the colony in a hollow log or at the base of a tree) in a new location each night (Swartz 2001, Chaves-Campos 2003). Due to this unreliability, obligate birds are reported to monitor approximately three colonies each day to ensure a consistent food supply. They are also known to rotate among colonies during the course of a day (Chaves-Campos 2011). Along with the movement of obligate bird among swarms, facultative birds join in and out of foraging groups creating the potential for high fluidity in the guild composition surrounding a swarm. These changes in size and composition of army ant-following birds over time, along with the mechanisms that control these dynamics have been little studied.

To better understand the group dynamics of birds that follow army ants, we sought to study the inter and intra-specific interactions in obligate and facultative birds in La Selva Biological Station, Costa Rica. We hypothesized that the dominance of obligate species drives emergent properties of the group such as size, composition, and patterns of change. The

rationale is that obligate bird species are highly invested in this system and therefore would incur the highest costs of interspecific competition. To minimize competition, obligate species might show niche partitioning among themselves, and aggressively displace facultative species (Calvie et al. 2000). If so, we would expect overall group size and composition to be regulated by obligate species. The abundance and species compositions of obligate birds would be constant, and those of facultative birds would fluctuate haphazardly. Another possibility is that small-scale change in facultative bird abundance at a foraging site would be controlled by corresponding changes in obligate bird abundance, but not vice versa.

#### METHODS

We conducted research at La Selva Biological Station, Costa Rica during 7-10 February 2018. On 8 February, we located one army ant swarm and made notes of associated bird species. We located and marked the bivouac and returned the next morning at 0630. On 9 February we located a second army ant colony and followed their trail to the head of the swarm.

#### RESULTS

We observed four species of birds during initial observations of the first army ant swarm—Ocellated Antbird (*Phaenostictus mcleannani*), Spotted Antbird (*Hylophylax naeviodes*), Northern Barred-Woodcreeper (*Dendrocolaptes sanctithomae*), and Plain Brown Woodcreeper (*Dendrocincla fuliginosa*). All four species have been described as obligate army ant followers (Garrigues and Dean 2014). However, although on 9 and 10 February we returned to and thoroughly inspected the forest near the ant bivouac, we saw neither ants nor ant-following birds in the area. It is likely that these ants were not foraging over these two days. Although we located a second swarm on the afternoon of 9 February, we saw no birds around these ants. On 10 February this colony moved into a section of the forest too dense to access.

#### DISCUSSION

As we were unable to collect the data that we aimed for, we devote the discussion of this

manuscript to elaborating as in a proposal the methods, possible results, and interpretations had we found active army ant and antbird systems. Our hope is to provide inspiration to subsequent researchers.

#### *Proposed Methods, Results, and Interpretation*

We would locate an army ant colony and its bivouac location for the night. In the morning we would arrive prior to any ant activity to ensure observation of all bird activity following ant emergence. Staying at particular positions relative to the head of the swarm, we would scan the area every five minutes, record the number and species of birds, and note whether these species are obligate or facultative. Between each scan we would select and observe one individual and record behaviors such as feeding, defensive displays (between or within species), and other dominant/submissive behavior. We would use previous research to catalogue these behaviors, such as Batcheller's (2012) descriptions of dominant and subordinate behaviors in Ocellated Antbirds.

To test whether group size is regulated by obligate birds, we would first visualize the change in total abundance of birds with time (Figure 1). We would expect to see the number of birds in the system increase as the size of the army ant foraging swarm increases and more insects are flushed out over a larger space. As the fan reaches maximum size, bird group size will saturate, as there is likely a maximum number of birds that can effectively forage with one swarm. Guild size could then be maintained by defensive behaviors of obligate species, as these species would be more likely to defend their positions within swarms due to their higher investment in feeding near the swarm. We would verify this by comparing the number of dominant and subordinate behaviors shown by obligate or facultative birds. If group size is not regulated by obligate birds, the total abundance of birds would show no pattern and obligate and facultative birds would show similar dominant/subordinate behaviors.

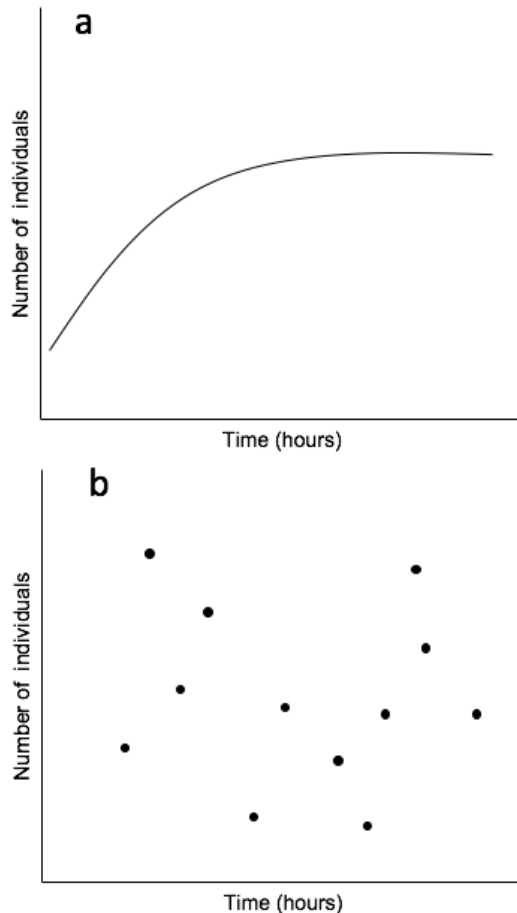


Figure 1. Two possible patterns for the relationships between time and number of individual birds following an army ant swarm. (a) initial growth in number of birds as swarm reaches its maximum size in which the bird group can benefit from the swarm. (b) number of individuals varies randomly over time.

Next, we would compare the abundance and species composition of obligate and facultative birds. Because of limited resources and dominance of obligate birds, we would expect a constant abundance of obligate individuals at a site (Figure 2). However, the abundance of facultative birds would fluctuate unpredictably throughout the day (Figure 2). It is important to note that there would be small scale fluctuations in obligate abundance as individuals move in and out of the system. To get a metric of species composition, we would use the ratio of the two most abundant species in each category. The composition of obligate bird species would be constant as a reflection of niche partitioning among obligate species (Figure 3). Niche

partitioning allows for individuals with different specializations to exploit the resource in different ways and thus minimizes competition. For example, woodcreepers can eat insects at higher locations in trees whereas antbirds forage closer to the understory, minimizing antagonistic interactions between the two. Facultative birds would not have distinct niches related to this system because they are opportunistic feeders, resulting in inconsistent compositions of facultative birds. Alternatively, the absence of consistent abundance and compositions of obligate birds could indicate a higher movement of birds between swarms than expected, giving rise to large fluctuations.

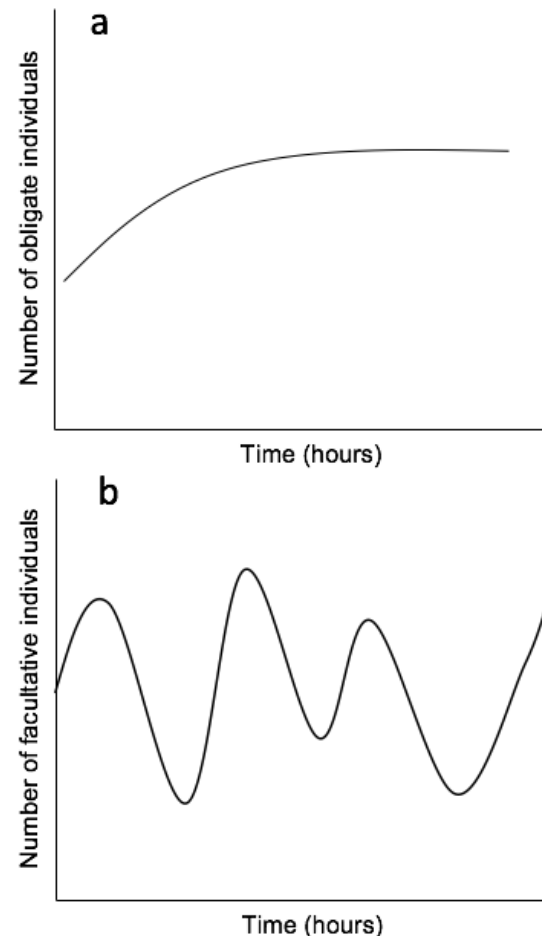


Figure 2. A possible result in which the number of bird individuals that are obligate followers of army ants increases initially and then reaches a stable state (a). Number of bird individuals that are facultative followers of army ants varies unpredictably (b).



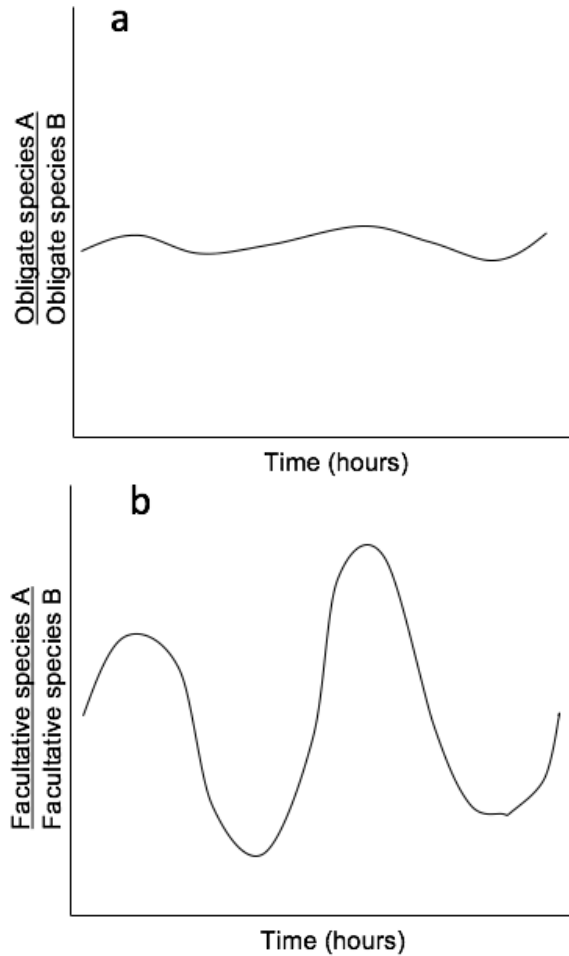


Figure 3. A possible result in which there is stability in the relative abundance of different species of birds that are obligate followers of army ants (a), and variability in the relative abundance of bird species that are facultative followers of army ants (b).

In addition to analyses conducted over a full day, we would look at smaller time scale changes. To understand the effects of obligate birds on facultative birds (and vice versa), we would compare the change in obligate bird abundance between two samplings to the change in facultative bird abundance in the next sampling interval (and vice versa). We would relate the change in number of both bird types. We would expect to see an inverse relation between change in obligate bird abundance and subsequent change in facultative bird abundance, as increase in obligate birds would lead to a decrease in facultative birds because of their subordinate status (Figure 4). A decrease in obligate birds could lead to an increase in

facultative birds as absence of obligates creates foraging opportunities requiring low investment on the part of the facultative individual. These momentary absences of obligate birds (due to factors such as movement between colonies) could be an important way in which facultative birds benefit from staying around army ant swarms despite their subordinate status. We would expect to see no effect of change in facultative bird abundance on obligate species due to obligate dominance.

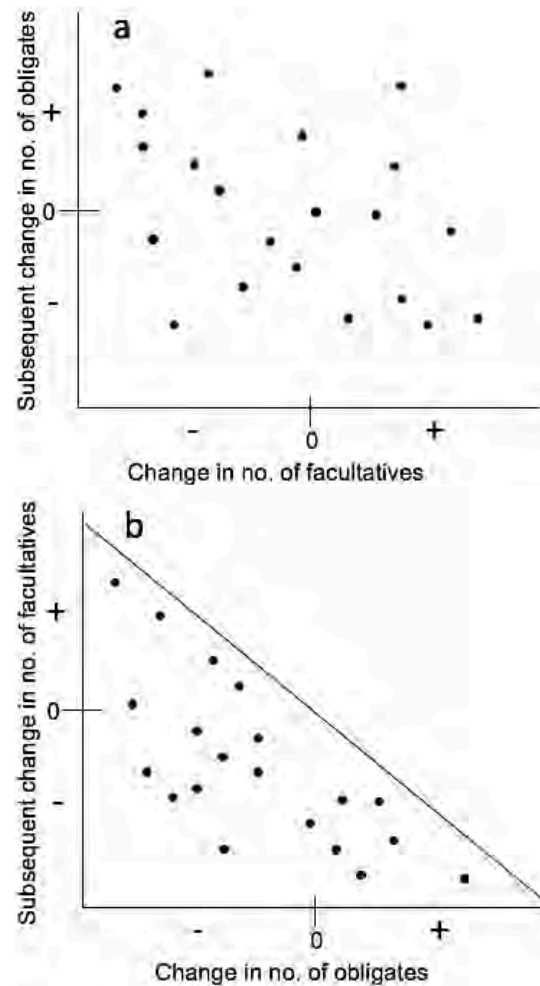


Figure 4. Two possible patterns in the flux of birds that are obligate followers of army ants vs facultative followers of army ants. On left, change in the number of facultative individuals does not affect subsequent change in the number of obligate individuals (a). On right, change in number of obligates does affect subsequent change of facultative individuals; with a decrease in obligates, facultative individuals may increase or decrease, but with an increase in obligates the number of facultative individuals will decrease (b).

The results of these analyses would provide a deeper understanding of the mechanisms for regulation of community structure of army ant-following birds. Many communities surrounding a shared resource are comprised of species with varying dependencies on those resources. Determining whether most shared-resource communities are dominantly regulated by the species most dependent on the resource (and therefore most invested in obtaining those resources) would provide a model with which to evaluate such systems.

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#### AUTHOR CONTRIBUTIONS

Both authors contributed equally.

#### LITERATURE CITED

- Calvie, M. S., M. K. Jennings, M. D. Foote, L. R. Nagy. 2000. Resource partitioning in army ant bird communities. *Dartmouth Studies in Topical Ecology* 2000, pp. 89-94.
- Batcheller, H. 2012. Ocellated Antbird. *Neotropical.birds.cornell.edu*. Published online at <https://neotropical.birds.cornell.edu/Species-Account/nb/species/oceant1/behavior>, accessed 2/12/18.
- Chaves-Campos, J. 2003. Localization of army-ant swarms by ant-following birds on the Caribbean slope of Costa Rica: following the vocalization of antbirds to find the swarms. *Ornitologia Neotropical* 14: 289-294.
- Chaves-Campos, J. 2011. Ant colony tracking in the obligate army ant-following antbird *Phaenostictus mcleannani*. *Journal of Ornithology* 152: 497-504.
- Brumfield, R. T., J. G. Tello, Z. A. Cheviron, M. D. Carling, N. Crochet, K. V. Rosenberg. 2007. Phylogenetic conservatism and antiquity of a tropical specialization: Army-ant-following in the typical antbirds (Thamnophilidae). *Molecular Phylogenetics and Evolution* 45: 1-13.
- Garrigues, R. and R. Dean. 2014. *Birds of Costa Rica*, Helm Field Guide, London, UK.
- Swartz, M. B. 2001. Bivouac checking, a novel behavior distinguishing obligate from opportunistic species of Army-ant-following birds. *The Condor* 103:629-633.
- Willis, E. O. 1983. Antbirds (Hormigueros, various genera). Pp. 546-547 in Janzen, D.H. (ed.). *Costa Rican natural history*. Univ. of Chicago Press, Chicago, Illinois.
- Willis, E. O., & Y. Oniki. 1978. Birds and army ants. *Annual Review of Ecology and Systematics* 9: 243-263.

## WHAT FACTORS INITIATE AND INFLUENCE *MONSTERA TENUIS* LIFE STAGES?

ANGELA E. ORTLIEB, CORINNE R. VIETORISZ, AND HANNAH I. HOFFMAN

Teaching Assistants: Michael B. Brown and Debora Goedert

Faculty Editor: Matt Ayres

**Abstract:** Phylogenetically-related individuals often share similar life histories, yet they can vary in the timing of and progression through their development. We evaluated factors that influence the life history of *Monstera tenuis*, a Neotropical epiphytic vine that undergoes three distinct maturation phases: growth without leaves, growth with immature leaves, and development of mature leaves. We found that vines did not begin producing immature leaves past 30 cm along the tree, suggesting that finite endosperm energy reserves necessitate a transition to autotrophy. We found that growing atop a conspecific plant during the immature life stage results in larger leaf sizes than growing atop a tree trunk. *M. tenuis* plants reached their mature stage within a precise height range; therefore, photon flux might be an ultimate cue that drives this life stage transition. We subsequently ruled out several possible proximal cues for the observed height range: period of time elapsed after starting the immature stage, a number of immature leaves produced, growth rate, size of immature leaves, and rate of immature leaf size increase. Temperature did not change along the growth substrate and thus likely does not instigate transitions between life stages.

**Key words:** epiphyte, La Selva, life cycles, *Monstera tenuis*

### INTRODUCTION

Life histories describe the stages through which organisms progress from inception through death. Although related individuals might progress through the same life history stages, the relative duration of these stages and the timing of transitions can vary significantly. Therefore, it is of interest to understand the mechanisms that drive certain developmental stages within species. Sometimes, stage transitions occur after a predetermined time or amount of development, but progression through life history does not rely entirely on an ontogenetic blueprint; rather, external factors might influence differential expression of general frameworks.

*Monstera spp.* (Araceae) offer a model system in which to investigate the mechanisms of life stage transitions. The genus contains 22 species that occur within the shade epiphyte community and have comparable life cycle stages (Madison 1977). First, seedlings are dispersed by birds and germinate on the ground, growing skototropically up to two meters in length on seed reserves until they encounter a tree. Once a plant encounters a tree, it ascends the trunk using the same reserves and develops immature leaves that are distichous and dorsiventral, flattened against the tree, and gradually increase in size up the length of the

tree trunk. As immature leaves develop, they begin as carbohydrate sinks, requiring energy-rich resources for growth. Once developed, these leaves become photosynthetically active and are carbohydrate sources that provide energy for further growth up the tree. The next developmental stage begins when the ascending shoot undergoes a morpho-physiological change to its adult form. The mature leaves are significantly larger, fan out from the host tree trunk, and have higher photosynthetic rates than the immature leaves (Janzen 1983).

Although all *Monstera* species pass through these same life stages, an adaptive radiation of species within the clade has involved the evolution of variant growth patterns (Madison 1997). We examined possible proximate and ultimate factors that control life stage transitions in *Monstera tenuis*. Because *M. tenuis* plants initially utilize resources provided by seed endosperm to grow towards and up a tree trunk, we predicted that immature leaves will begin below a certain height, as the plant must initiate photosynthesis to generate its own nutrients after depleting finite endosperm reserves (Madison 1977). Another factor that could influence *M. tenuis* immature life stages is the presence of conspecifics acting as a substrate. Leaves differ from tree bark in texture, nutrient content, and

other factors that could influence growth of a subsequent *M. tenuis* either positively or negatively. Growing, immature plants might exploit the surface or nutrients of a conspecific for its own attachment and growth, or perhaps roots cannot attach to this surface as effectively such that more nutrients must be allocated to maintaining an adequate root system.

We also considered prospective cues from the immature to the mature life stage in *M. tenuis*. Possible thresholds that an *M. tenuis* plant might have to reach to begin developing mature leaves could involve height, growth rate, size of immature leaves, and rate of immature leaf size increase. All of these physiological traits could depend on environmental conditions and thus might serve as signals for when an environmental condition reaches an optimum for the mature stage to occur.

Lastly, McNaughton et al. (1985) cited temperature as a primary factor affecting plant progression through developmental stages, as photosynthetic efficiency increases with increasing temperature to a certain point. Given that the temperature of a tree trunk generally increases with height, we predict that life stage transitions might occur once *M. tenuis* plants reach a certain height on a tree and thus a certain temperature (Rijkers et al. 2000).

## METHODS

We located *M. tenuis* along trails at the La Selva Biological Research Station in the Caribbean lowlands of northern Costa Rica. For each plant, we recorded as many of the following traits as possible to gather information about developmental stages: height at which the immature leaves began on the trunk, whether or not the immature leaves grew on top of an older plant, and the height at which the immature leaves ended and/or the mature leaves began. For heights that were too tall to measure with a ruler, we used a pole marked every five centimeters. We measured the length and width of as well as the internodal distance between five immature leaves that were roughly four feet from the bottom of the tree. We collected the same information for the five immature leaves that occurred directly before the beginning of the mature leaves. When we could not measure leaf

traits with a ruler, we aligned a ruler attached to a pole along the focal length and analyzed a photograph of this arrangement for the relevant information. We used the formula for the surface area of an ellipse to estimate leaf size.

### *Determination of transition to immature leaves*

We created a frequency distribution of the heights at which immature leaves began to develop to evaluate whether this trait is associated with the maturity transition.

### *Factors influencing growth in the immature stage*

To determine whether ascending on top of a conspecific influences the growth patterns of an *M. tenuis* plant, we used a paired t-test comparing the leaf area of the newer plant with that of the leaf growing directly underneath it.

### *Determination of transition to mature leaves*

*Height of transition to mature leaves.* To evaluate the possible association between the height of an *M. tenuis* plant and the point at which immature leaves begin to develop, we created a frequency distribution of these height values.

*Growth rate.* Using internodal distance as a proxy for growth rate, we ran a nested ANOVA to determine whether more growth rate variation existed between *M. tenuis* or within individual plants. To evaluate the relationship between growth rate and the height at which an *M. tenuis* plant reaches the mature stage, we used a linear regression to compare the average internodal distance of plants to the heights at which they developed mature leaves.

*Size of the last immature leaf.* To evaluate the relationship between the size of the last immature leaf and the height at which an *M. tenuis* plant reaches the mature stage, we used a linear regression to compare the areas of the last immature leaves to the heights at which they developed mature leaves.

*Change in immature leaf sizes just before maturity.* To investigate the potential association of growth patterns with the transition from immature to mature leaves, we calculated the percent area increases between the last five successive leaves of plants that had matured; we

subsequently averaged these four values as a proxy for change in immature leaf size just before maturity. To evaluate the relationship between the leaf size changes just before maturation and the height at which an *M. tenuis* plant reaches the mature stage, we ran a linear regression comparing these values for plants to the heights at which they developed mature leaves.

#### *Temperature as an environmental cue for maturity transition*

To evaluate whether temperature change along tree trunks could affect *M. tenuis* life stages, we recorded bark temperature at the bottom of the tree and at the tallest height of the plant using a temperature gun.

## RESULTS

We measured 54 *M. tenuis* plants. On some plants, we could not measure immature leaves near the base because they had degraded. On other plants, we could not record attributes of the last immature leaves because they had not yet developed mature leaves.

#### *Determination of transition to immature leaves*

Most *M. tenuis* (87%) produced their first immature leaf within the lower 30 cm of the tree trunk (Fig. 1).

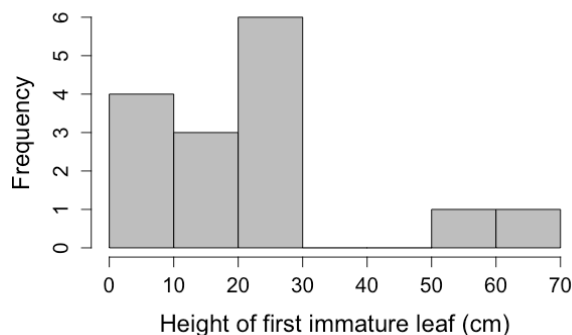


Figure 1. Frequency distribution of heights of the first immature leaf in *M. tenuis*. (N=15 plants)

#### *Factors influencing growth in the immature stage*

We found six instances in which *M. tenuis* climbed their host tree on top of an older *M. tenuis* stem. In some cases, they grew atop a single older vine even though most of the trunk

had no vines. In these cases, the younger *M. tenuis* had larger leaves ( $\bar{x}$  = 233% larger) than the conspecific on which it grew (paired-t = 3.26,  $P$  = 0.02,  $df$  = 5).

#### *Determination of transition to mature leaves*

*Height of transition to mature leaves.* For most *M. tenuis*, the transition from immature to mature leaves occurred at 3.0 to 4.1 m (70% of 16 plants). The lowest and highest heights of transition were 2.1 and 5.1 m.

*Growth rate.* Within individual *M. tenuis* plants, internodal length was quite constant. However, there was conspicuous variation in internodal lengths among plants, ranging from 5.0 to 12.0 cm (95% of the total random variance in internodal length was among plants, with only 5% within individual plants). The average internodal distances of *M. tenuis* plants were unrelated to the heights at which leaves transitioned from immaturity to maturity (slope  $\pm$  SE =  $5.96 \pm 16.14$ ,  $P$  = 0.72,  $r^2$  = 0.02).

*Size of the last immature leaf.* The areas of the last immature leaves ranged from 241.9 to 1134.1 cm<sup>2</sup> (mean  $\pm$  SD =  $648.5 \pm 291.7$  cm<sup>2</sup>). The size of the last immature leaf was unrelated to the height at which leaves transitioned from immaturity to maturity (slope  $\pm$  SE =  $-0.06 \pm 0.08$ ,  $P$  = 0.47,  $r^2$  = 0.04).

*Change in immature leaf sizes just before maturity.* Average percent increase in leaf area between the last five successive leaves before maturity transition ranged from 2.7 to 69.7 % (mean  $\pm$  SD =  $24.51 \pm 16.48\%$ ). This increase was unrelated to the height at which leaves transitioned from immaturity to maturity (slope  $\pm$  SE =  $0.35 \pm 1.36$ ,  $P$  = 0.80,  $r^2$  = 0.01).

#### *Temperature as an environmental cue for maturity transition*

There was little temperature difference between the base of the trunk on which an *M. tenuis* grew and the bark near the top of the plant (mean difference (top - bottom)  $\pm$  SE =  $0.61 \pm 0.31$  °C).

## DISCUSSION

This study provides insights into factors that influence *M. tenuis* life stages.

*Determination of transition to immature leaves*

Viable *M. tenuis* might produce immature leaves before a certain threshold due to the fact that the plants rely solely on endosperm resources before creating leaves. This source contains a finite amount of nutrients; therefore, if a plant does not photosynthesize before or soon after consuming these nutrients, it likely will not survive (Madison 1977). The modest deviations in leaf start heights could indicate variation in endosperm size or differences in seed dispersal and germination distances from trees.

*Factors influencing growth in immature stage*

Both the fact that immature *M. tenuis* growing atop conspecifics develop leaves larger than those supporting them and our observation that *M. tenuis* grew atop conspecifics even when there was available bark space on the same tree suggest that plant substrate confers an environmental advantage that trunk substrate does not. Perhaps *M. tenuis* roots are better able to extract nutrients from conspecifics than from tree bark, or perhaps the old plant provides a more suitable substrate for attachment.

*Determination of transition to mature leaves*

The fact that a large majority of *M. tenuis* transitioned to mature leaf production within a one-meter height range suggests a strong selective pressure for transition at this height. In accordance with previous findings that light availability is not associated with life stage progression in this species (Coleman et al. 2002), *M. tenuis* might instead be driven by an ultimate environmental cue for light availability, transitioning at a height that generally provides sufficient photon flux. If so, plants must rely on proximate cues to estimate when they have reached this predetermined height. The finding that plants with greater internodal distances did not reach maturity higher on their hosts rules out two possible proximate cues: that plants produce a certain number of leaves before maturing and that plants grow for a certain amount of time before maturing. If plants did produce a set number of immature leaves, ones with greater spacing between those leaves would reach maturity higher on the tree. If plants remained in the immature stage for a set period of time, ones

that grow faster would reach maturity higher on the tree. The finding that internodal lengths – and thus growth rates – remained constant for a plant just before this transition and the finding that these lengths varied significantly across individuals rules out the possibility that *M. tenuis* use growth rate as a proximal cue for height, as growth rate does not seem to change during the immature stage, and plants did not have to maintain a particular growth rate (i.e., reach a particular internodal distance) before maturing. Given that the size of the last immature leaf just before this transition was unrelated to the height at which mature leaves began, this physiological trait also does not seem to be a proximal cue for height of transition to maturity. One other factor that does not emerge as a proximate cue for height is the average increase in leaf size just before maturity, as this trait did not correspond with transition height. Although this study excludes five possibilities, it remains unclear what proximal cues *M. tenuis* use to reach the precise height range at which they mature.

*Environmental cues for maturity transitions*

Because temperature did not fluctuate significantly along trees with *M. tenuis*, this environmental factor likely does not contribute to either life stage transition, as transitions occurred within particular height ranges. However, it is possible that slight temperature fluctuations result in variation within life stages, such as germination, growth rate, and leaf development (Roberts 1988).

Although an organism's life history provides a framework for its ontogeny, this study serves as a reminder that external factors can cause a great deal of variation and that life histories exhibit plasticity even within species that occupy the same habitats. It is also notable that the external factors generating this plasticity cannot necessarily be correlated with conspicuous morphological discrepancies. Finally, there does not always exist an optimal pattern with which species progress through their life histories; rather, the ability to deviate from a rigid mold and develop both randomly and in a wide variety of contexts could itself be an optimal strategy.



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AUTHOR CONTRIBUTIONS

All authors contributed equally.

LITERATURE CITED

- Coleman, L. M., Kristin S. Nowak, and Robert. F. Rogers. 2002. *Monstera*, the early years. Dartmouth Studies in Tropical Ecology 2002, pp. 138-141.
- Madison, M. 1977. A revision of *Monstera* (Araceae). Cambridge, MA: Harvard University Press.
- McNaughton, K., P. Gandar, and H. McPherson H. (1985). Estimating the effects of varying temperature on the rate of development of plants. *Annals of Botany* 5: 579-595.
- Ray, T., and J. M. Savage. 1983. Species accounts: *Monstera tenuis* (Chirravaca, Mano de Tigre, Monstera). Pages 278-280 in D.H. Janzen, editor. Costa Rica Natural History. University Press of Chicago, Chicago, IL.
- Rijkers, T., Pons, T. L., & Bongers, F. (2000). The effect of tree height and light availability on photosynthetic leaf traits of four Neotropical species differing in shade tolerance. *Functional Ecology* 14:77-86.
- Roberts, E.H. (1988). Temperature and seed germination. *Symposia of the Society for Experimental Biology* 42:109-132.







## AN EVALUATION OF MANAGEMENT STRATEGIES ON QUEEN CONCH BASED ON MOVEMENT

Project initiated by: Celia Chen

Conducted by Dartmouth Biology FSP 2018

Written by: PAUL J. VICKERS, ALEXANDRA M. STENDAHL, CLAYTON E. JACQUES, AND ALEXANDER W.  
COTNOIR

Teaching Assistants: Debora Goedert and Michael B. Brown  
Faculty Editor: Celia Chen

**Abstract:** Little Cayman has had marine protected areas in effect for over 30 years in an attempt to protect the coral reef ecosystems surrounding the island. Queen conch (*Lobatus gigas*) is one of the most economically and culturally important fisheries in the islands; however, the population of the long-lived gastropod is collapsing throughout the Caribbean. We evaluated the impact of the conch replenishment zone bordering Owen Island in South Hole Bay of Little Cayman through transect surveys and observations of conch movement. In 24 20-meter transects in both the harvest and replenishment zone and on both sand and coral rubble substrate within each zone we found greater density of conch in the replenishment zone than the harvest zone. In the replenishment zone, more conch were present in coral rubble substrate than sand substrate. In the harvest zone, no substrate preference was displayed. In a survey of movement ability, conch moved an average of 29 meters per day which is much greater than previous studies indicate. This large movement range indicates a strong potential of conch to cross between harvest and replenishment zone. Based on our findings, we recommend that the harvest season be shortened in addition to expanding the MPA to include the most favorable habitat to best protect the species.

### INTRODUCTION

In response to global targets set under the Convention on Biological Diversity, the number of marine protected areas (MPAs) has rapidly increased (Edgar et al. 2014). However, the effectiveness of these areas for conservation is unclear. In the Caribbean Sea, many areas face exceptional pressure on the delicate marine ecosystems due to a reliance on fishing and rapid increase in tourism. Islands such as the Cayman Islands have responded by establishing MPAs. Such areas have been established in Little Cayman and Cayman Brac for 32 years (Dromard et al. 2011). However, based on surveys of fish density and biomass done by Dromard et al. in 2016, the MPAs showed mixed success on Cayman Brac, the MPA did not impact fish density, however there was a positive impact in Little Cayman (Dromard et al. 2011). Queen conch and other non-fish taxa were not measured in this extensive survey.

Queen conch (*Lobatus gigas*) is a large marine gastropod found in the Caribbean Sea and adjacent areas of the Atlantic Ocean. Conch are frequently harvested as seafood, and have

experienced population declines ostensibly from overharvesting (Kough et al. 2017). After widespread declines were observed in Honduras, Haiti, and the Dominican Republic, queen conch was listed under Annex II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1992. Despite conch trade restrictions placed upon the aforementioned countries, queen conch continue to be harvested in several Caribbean countries that have implemented sustainable management plans (SMPs), including the Cayman Islands (Williams 2003). To mitigate population declines, officials in the Cayman Islands established coastal “replenishment zones” in which the harvest of conch is prohibited. One such replenishment zone is located in South Hole Sound, on the southern side of Little Cayman Island.

The queen conch population is exceptionally vulnerable due to their late sexual maturity (often 3.5 years), ease of hunting, and density dependent reproduction. Juvenile conch are exceptionally vulnerable to predation by rays, sharks and grouper. Conservation efforts which

first focus on predator species such as grouper can increase conch juvenile mortality. Kough et al. (2017) speculates that this could prevent the recovery of a conch population. Lastly, conch habitat is not created equal. The juveniles seem to prefer a habitat uniquely different than adults; however, this variation in habitat occupation across age classes is not considered during the creation of MPA. Moreover, larvae can disperse across large distances, and establish themselves outside of the protective boundary (Kough et al. 2017).

To begin evaluating the effectiveness of the queen conch replenishment zone, we performed a general population survey, as well as a study of conch movement. We hypothesized that conch populations are greater in the replenishment zone, and based on local fisherman knowledge, that conch abundance is greater in coral rubble substrate. To evaluate conchs' abilities to cross the MPA boundary, we investigated conch movement. We compare our findings with previous reports that conch will move around 11 meters a day as found in Doerr and Hill 2013.

## METHODS

### *Conch distribution*

To determine distribution of queen conch at sites with different harvesting pressures and substrate types, we established 24 transects in South Hole Sound in Little Cayman Island (Figure 1). The Sound includes a protected replenishment zone for queen conchs, and a harvestable zone, both of which include areas with sandy and coral rubble substrates. We established 12 transects in the harvest and replenishment zones, between 250 - 350 m from the replenishment zone boundary (Figure 1). In each zone, we divided transects equally between sandy and coral rubble substrate, resulting in 3 sets of 20 m transects per zone and substrate. Each pair of transects was spaced 50 meters apart.

To define each transect, two researchers stretched a 20 meter transect tape. Using snorkeling gear, other researchers swam the length of the transect, gathering conch within 2 meters on either side of the line. We scored each shell found as live conch, empty shell or harvested shell (characterized by empty shells

with clear signs of the cut used to detach the animal), and measured shell length, and shell flange thickness (Figure 2). We determined age class of based on flange thickness: a shell with a thickness of 10 mm or above indicated sexually mature individuals (Stoner et al. 2012; Doerr and Hill 2013).

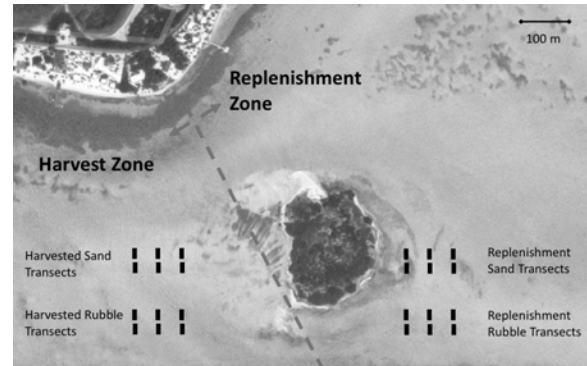


Figure 1. Map of transects used to measure conch distribution in South Hole. Each black line represents a transect. The red dashed line represents the boundary of the conch replenishment zone.

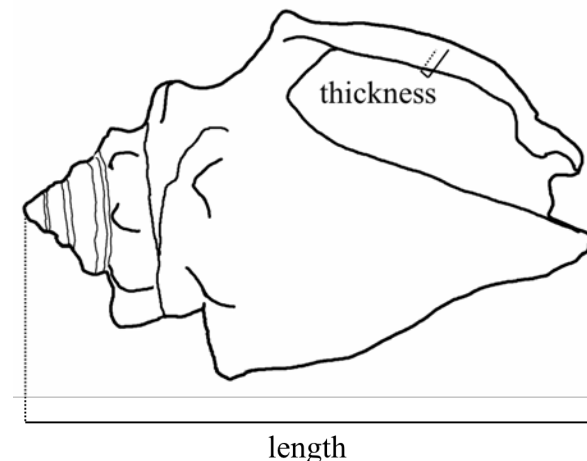


Figure 2. Dimensions measured on each Queen Conch.

### *Conch movement*

We attached numbered buoys to five conchs on the afternoon of Feb. 18 and deployed a corresponding labelled buoy (anchored to a rock) beside each conch. All conch were tagged on vegetated rubble substrate. For each conch, we measured lip thickness at the halfway point along the flange and total shell length, determining age class as previously described. We measured linear displacement overnight

(approximately 16 hours), based upon distance between conch from the corresponding attached buoy. On the morning of Feb. 19, we attached numbered buoys to 5 different conch on vegetated rubble substrate. We returned 6 hours later and measured linear displacement as well as direction travelled in relation to the direction of the prevailing current.

## RESULTS

### *Transect Survey*

We found 187 conch shells across the 24 transects. Of these shells, 160 represented live conch, 11 were empty most likely due to death of organism by natural causes or predation, and 16 had been harvested.

After log-transforming the data to satisfy assumption of normality, the number of conches per transect differed between based on substrate and zone (Figure 3;  $F_{2,21} = 6.78$ ,  $P < 0.05$ ). In the harvest zone, we found no difference in the number of conches per transect between the sand and coral rubble substrates ( $F_{2,21} = 0.31$ ,  $P = 0.58$ ). We found more conches per transect in the replenishment zone compared to the harvest zone ( $F_{2,21} = 11.13$ ,  $P < 0.01$ ). In the replenishment zone, more conches were found in coral rubble than sand ( $F_{2,21} = 8.65$ ,  $P < 0.01$ ). An equal proportion of juveniles was found in both substrates within the replenishment zone (Figure 4). Four times as many juveniles were found in the rubble substrate of the replenishment zone than any transection group.

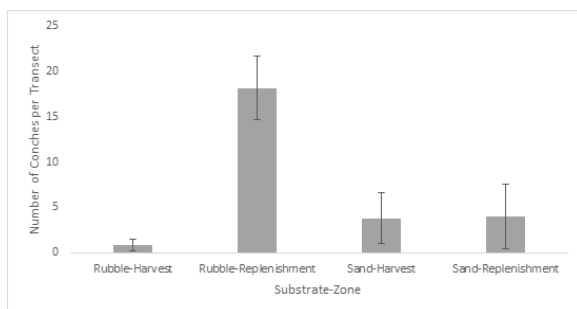


Figure 3. Number of conches measured per transect (mean  $\pm$  SE) by substrate type and conservation zone.

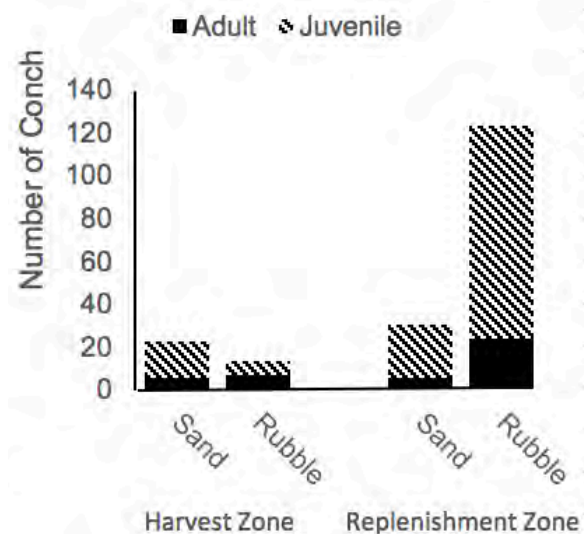


Figure 4. A comparison of age class between the harvest and replenishment zone and the substrates within each zone.

### *Movement Survey*

We surveyed the movement of ten conch: five juveniles and five adults. Conch shell flanges ranged in thickness from 1.0 to 13.6 mm (mean  $\pm$  SE =  $7.5 \pm 1.8$ ) and in length from 17.0 cm to 23.0 cm (mean  $\pm$  SE =  $20.0 \pm 5.6$ ). Conchs moved between 0.30 to 2.52 m/hr (mean  $\pm$  SE =  $1.21 \pm 0.25$  m/hr). These values extrapolate to a range of 7.14 to 60.50 m/day. Individuals did not move significantly faster overnight or during the day ( $t = 0.66$ ,  $P > 0.05$ ,  $df = 8$ ). Conch shell length had no impact on movement rates (slope  $\pm$  SE =  $-0.01 \pm 0.02$ ,  $P = 0.58$ ,  $r^2 = 0.04$ ). Similarly, shell thickness did not move at higher rates (slope  $\pm$  SE =  $0.04 \pm 0.05$ ,  $P = 0.36$ ,  $r^2 = 0.10$ ). Adults and juveniles did not move at significantly different rates ( $t = 1.01$ ,  $P > 0.05$ ,  $df = 8$ ).

## DISCUSSION

Our results indicate that there is a greater abundance of conch in the replenishment zone, suggesting that the zone may be successfully protecting conch. Our movement study found that the average conch moved approximately 30 meters a day with some traveling upwards of 60 meters per day. This average displacement is greater than the maximum daily distance found by Doerr and Hill (2013) tracking conch in the Virgin Islands. The divergence in estimated

distances may be due to substrate differences or other abiotic conditions such as wind, current, or temperature. Further studies could track conch using GPS tracking similar to Doerr and Hill (2013) to get more comparable results.

Because conch can move large distances, MPA might not be the best methodology to protect the organisms. While our data show that there is a higher abundance in the replenishment zone, conch can easily cross this line into the unprotected zone. A reduction of harvest season length may help protect conch who may have otherwise crossed over the line during the current three-month harvest season. Future study could investigate motivations behind movement such as searching for a mate or food resource. Additionally, study of conch home range could identify conch hotspots most in need of protection.

In 2012, a similar survey of South Hole Sound discovered higher numbers of both juvenile and adult conch in the replenishment zone than in the harvesting zone (Chowdhury et al. 2012). They found a higher proportion of younger conch in the replenishment zone and created a model of future population size based on this distribution. The model predicted that the population in the protected area would grow more quickly than the population in the harvested zone, which may be experiencing a slower growth rate due to Allee effects. Chowdhury et al. (2012) concluded that the significant differences in density in sites so near to each other supported the definite effectiveness of the replenishment zone and suggested that the conch were largely not moving across the boundary line. Our corollary study of conch movement, however, revealed that these animals may move far more than Chowdhury et al. predicted. This implies a higher rate of conch movement both into and out of the harvest zone. Regardless of this result, we again found higher numbers of conch in the replenishment zone than in the harvested zone.

By synthesizing the results of multiple years of FSP study, we can conclude that conch

have strong substrate preferences. Additionally, certain habitats, such as coral rubble, may function as nursery habitat and be critical to species conservation (Kough et al. 2017). In order to best manage this fragile fishery, we recommend the implementation of standardized population surveys. Additionally, further study could try to address shift in age class composition and the role different reef areas play in recruitment. Lastly, we recommend that the Island consider shorten the take season, in addition to implementing size class restrictions and increasing MPA size, to compensate for the ability of the conch to transverse large distances and best protect these charismatic organisms.

#### LITERATURE CITED

- Andrew S. Kough, Heather Cronin, Rachel Skubel, Carolyn A. Belak, Allan W. Stoner. 2017. Efficacy of an established marine protected area at sustaining a queen conch *Lobatus gigas* population during three decades of monitoring. *Mar Ecol Prog Ser.* 573: 177–189.
- Chowdhury MHR, AL Deffebach, JT Reib. 2012. Replenishment Zones are Effective for Conservation of *Strombus gigas*. *Dartmouth Studies in Tropical Ecology* 2012:159.
- Doerr, J. C. and R. L. Hill. 2013. Home range, movement rates, and habitat use of queen conch, *Strombus gigas*, in St. John, U.S. Virgin Islands. *Caribbean Journal of Science* 47:251-259.
- Dromard, C. R., McCoy, C., Turner, J. R. 2011. Measuring the Performance of Marine Protected Areas: The Case of Little Cayman and Cayman Brac, Cayman Islands. *Proceedings of the Gulf and Caribbean Fisheries Institute*.
- Edgar, G. J., Stuart-Smith, R. D., Willis, T. J. . . . et al. 2014. Global conservation outcomes depend on marine protected areas with five key features. *Nature* 506(7487), 216-20
- Stoner AW, KW Mueller, NJ Brown-Petersen, MH Davis and CJ Booker. 2012. Maturation and age in queen conch *Strombus gigas*: Urgent need for changes in harvest criteria. *Fisheries Research.* 131-133: 76-84.
- Williams, Michael. 2003. CITES suspends trade in queen conch shellfish. U.N. Environmental Programme.



# *ACROPORA CERVICORNIS* STRUCTURE AS A DETERMINANT OF REEF FISH ABUNDANCE AND DIVERSITY

ALEXANDER W. COTNOIR, ALEXANDRA M. STENDAHL, ALEC B. COBBAN

Teaching Assistants: Michael B. Brown and Debora Goedert  
Faculty Editor: Celia Chen

**Abstract:** Coral reefs are among the most biologically diverse ecosystems in the world, and yet these important habitats have experienced many challenges stemming from climate change, overfishing, and pollution. Restoration of coral reefs through outplanting has shown recent promise to increase abundance of endangered coral species as well as structural complexity of reefs, which in turn may impact the diversity and abundance of reef fishes. Recently, staghorn coral (*Acropora cervicornis*) outplantings have been established around Little Cayman, but such efforts require considerable time and money, which make it necessary to understand the importance of the interactions between corals and fish communities. This study uses visual point surveys to assess fish communities at two pairs of corresponding patch reefs containing and lacking stands of *A. cervicornis*. We show a significant increase in total fish abundance and species diversity in the presence of *A. cervicornis* in both Grape Tree Bay and Preston's Bay, suggesting that the presence of *A. cervicornis* provides an attractive habitat for a variety of fish assemblages. We also find that average abundances of ecologically important fish species such as parrotfish and damselfish are significantly greater at sites with *A. cervicornis*, suggesting that *A. cervicornis* may serve as an attractive habitat for both functional groups. Parrotfish are important algal grazers, helping to maintain coral colonies, although damselfish can have a range of negative effects on coral, thus inviting future research on the broader effects of the damselfish assemblages attracted to *Acropora* stands. Our surveys also suggest that a recently outplanted *A. cervicornis* site supports greater fish diversity and abundances than a corresponding patch reef lacking staghorn corals, suggesting that these man-made stands still offer considerable ecological benefit.

**Key words:** *Acropora cervicornis*, coral outplanting, fish community composition, reef structure

## INTRODUCTION

Coral reefs support some of the greatest diversities of organisms of any ecosystem across the globe. Coral diversity and abundance have declined significantly in recent decades due to the combined effects of disease, loss of large herbivores, coastal development, and climate change, resulting in the disturbance of associated reef communities. As studies have recently proposed, the future of these ecosystems depends not only on their continued protection from coastal development and fishing pressures, but also upon active management of coral reefs (Hughes et al 2017).

Active reef management can take many forms, from targeted removal of invasive species, to coral outplanting, the cultivation and subsequent planting of corals to wild reef systems. Outplanting has shown recent promise within the Cayman Islands. Efforts around Little Cayman have focused on cultivating and planting stands of the critically endangered staghorn coral (*Acropora cervicornis*) which

experienced a population collapse across the Caribbean in the 1980s. Given the substantial amount of labor, expense, and time needed to raise and install such outplantings, understanding how this coral species affects reef ecosystems and how artificially-planted stands might differ from wild stands is of utmost importance.

The habitat that corals provide can directly affect fish populations. Live coral coverage significantly impacts both fish abundance and diversity (Bell and Galzin, 1984). As previous studies suggest, structural complexity of reefs is considered to be a primary factor shaping reef fish assemblages (Graham & Nash 2013). The loss of structural complexity with the disappearance of branching coral species such as *A. cervicornis* may thus have far-reaching impacts on the diversity and abundance of particular groups within fish assemblages. The effect of coral on reef fish populations can occur because of the variety of structurally complex habitats produced by corals; for example,

increased rugosity and holes among branching corals may provide critical refuges for juvenile fishes.

Healthy reef fish populations are important for successful coral recruitment and growth. Specific functional groups of fish are of considerable importance to reef ecosystems due to their respective foraging behaviors. For example, parrotfishes comprise one of the primary algae consumers in reef ecosystems, opening space for coral recruitment and growth (Brock 1979). Conversely, damselfish tend to support algae growth by creating territories that exclude grazers and via algal farming activities displayed in some species (Sammarco 1983). Considering how different functional groups of reef fishes can positively or negatively affect reef-building corals, understanding which fish species associate with different coral structures can be helpful to better inform management of reef ecosystems.

In this study, our objective was to determine how stands of *A. cervicornis* affect the diversity and abundance of local reef fish assemblages. We asked three primary questions. First, how does fish diversity and total abundance change with presence/absence of *A. cervicornis*? We hypothesized that fish diversity and abundance changes between sites with and without *A. cervicornis*. We predicted that sites with *A. cervicornis* exhibit higher fish diversity, and a higher total number of fish, due to the greater structural complexity created by *A. cervicornis* thickets. Next, we asked whether abundances within specific fish families or species change in sites with and without *A. cervicornis*. We hypothesized that the local abundance of individual fish species changes between sites with and without *A. cervicornis*. We predicted that sites with *A. cervicornis* have a higher abundance of fish than sites without *A. cervicornis*. Finally, we asked whether fish diversity and total abundance differ between natural thickets and outplanted thickets of *A. cervicornis*. Understanding how *A. cervicornis* impacts fish diversity and abundance, both on the level of functional groups and at the species level will allow us to better predict how reef fish will be affected by the absence or presence of *A. cervicornis* corals, and how outplantings may alter reef ecosystems.

## METHODS

### Site selection

We conducted fish surveys at 4 sections of patch reef, within the fringing reef surrounding Little Cayman Island. Two sites were located on the north side of the island in Grape Tree Bay and two sites were located on the south side at Preston Bay. At each of two reef sites, we compared areas with *Acropora* stands against areas without (Figure 1, Figure 2). We surveyed the largest stand of outplanted *A. cervicornis* (planted in August 2016) in Grape Tree Bay, as well as a structurally similar site that lacked *A. cervicornis* in Preston Bay. In Preston Bay, we surveyed a wild stand of *A. cervicornis*, alongside a similar site that lacked *A. cervicornis*. The corresponding non-*A. cervicornis* sites on each side of the island were located 10 meters away from the *A. cervicornis* sites. The non-*A. cervicornis* sites contained live corals (i.e.- finger corals and boulder corals) which provided similar structure as the corals growing alongside the *A. cervicornis* at the outplanted site.

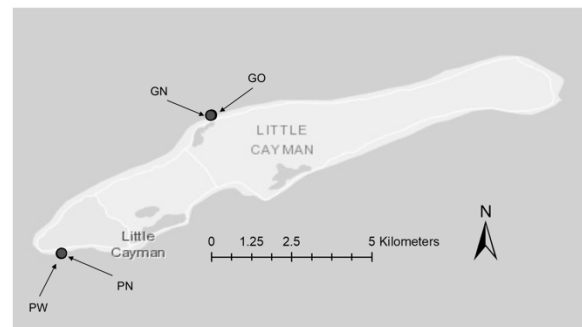


Figure 1: Map of sampling locations at Grape Tree Bay (GN and GO) and Preston Bay (PN and PW) surrounding Little Cayman. GN = Grape Tree Bay, no *A. cervicornis*; GO = Grape Tree Bay, *A. cervicornis* outplanting; PW = Preston Bay, wild *A. cervicornis*; PN = Preston Bay, no *A. cervicornis*.



Figure 2: a: site with outplanted *A. cervicornis* in Grape Tree Bay; b: corresponding non-*A. cervicornis* site in Grape Tree Bay; c: site with wild *A. cervicornis* in Preston Bay; d: corresponding non-*A. cervicornis* site in Preston Bay.

#### Measuring site characteristics

At each *A. cervicornis* stand (outplanted on the north side of the island; wild on the south side), we measured the length and width of the thicket, the water depth at mid-tide, and used ongoing survey data from CCMI to determine the average size of coral colonies (where size of *A. cervicornis* is measured as total linear extension [TLE], which consists of the sum of the length of all of the branches of an individual colony). We used these measurements when choosing comparable non-*Acropora* sites on either side of the island, and to evaluate structural differences between the wild *A. cervicornis* stand in Preston Bay and the outplanted stand of *A. cervicornis* in Grape Tree Bay. Additionally, we counted the number of individual *A. cervicornis* colonies, and took photographs from above the coral stand to quantify percent cover of *A. cervicornis* and density. At both sites, we held an eighth-square meter PVC quadrat over two representative sections of coral and took digital photographs from above using a Canon PowerShot D30 camera. We then quantified the percent *A. cervicornis* coverage as the proportion of total area of the PVC quadrat using ImageJ software, averaging across the two photographs to provide a better estimate of coral cover (Figure 3). To determine the location of each site within the general reef, we took GPS coordinates at each site (Figure 1). We then used ArcGIS 10.4.1 to measure the distance of each site to the edge of the main fringing reef, as well as to the shore.

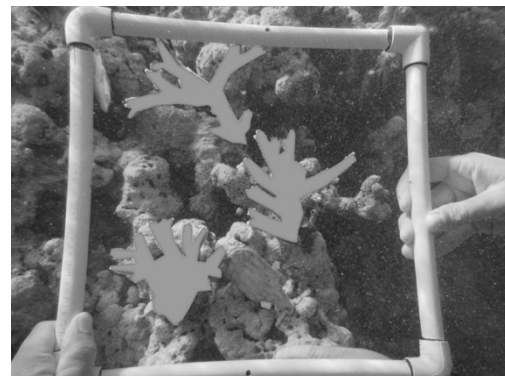


Figure 3. Process of categorizing percent *A. cervicornis* cover using ImageJ software. Non-colored areas represent underlying or dead corals. Percentage *A. cervicornis* was calculated as the number of pixels out of the entire number of pixels inside the PVC frame.

### Fish surveys

We conducted periodic counts of fish visible at each of the four reef sites using a point count method modified from Bohnsack & Bannerot (1986). Before conducting each fish count, we waited two minutes to allow fish to acclimate to our presence. We scanned a 1 m<sup>3</sup> cube survey area within the center of the patch of corals at each site, taxonomically classifying and counting all the individual fish that crossed within the volume in one minute of scanning (only counting each fish once per count). We conducted a total of 20 fish counts at each of the four sites in the manner described above, rotating observers to minimize observer bias. We also conducted an equivalent number of point counts in the mornings and afternoons in order to minimize any impacts of time of day/tide on fish assemblages.

### Statistical analysis

We performed all statistical analyses in JMP Pro 13.0. As a measure of fish diversity, we used the Shannon-Weiner Diversity Index:

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

where: H = the Shannon diversity index, S = total number of species encountered, and  $p_i$  = fraction of the entire population made up of species  $i$ . After calculating Shannon-Weiner Index values for each survey bout, we averaged across all 20 survey counts to get an average site diversity. All fish observation data met assumptions of normality under a Shapiro Wilk test and equal variance under a Bartlett's test. We used a one-way ANOVA and post hoc Tukey's HSD test to compare average diversity of fish assemblages across sites, as well as a set of two sample t-tests to compare the average diversity of fish assemblages between sites with and without *A. cervicornis*. We also performed a one-way ANOVA and post hoc Tukey's HSD test to compare the mean total fish abundance, as well as the mean abundances per species (calculated for each survey bout across sites). We chose fish species to compare as those that were both most abundant and of significant ecological importance for the maintenance of reef ecosystems.

## RESULTS

### Site characteristics

While the size of the individual *A. cervicornis* colonies in each site were estimated to be the same (TLE average = 73.6 cm) as was the water depth, there were several key differences between the sites with staghorn corals. The outplanted thicket had twice as many coral colonies as the wild thicket and covered a larger area. The wild thicket had a much higher density of coral, in both coral/meter, as well as percent coral cover. The other major difference was the location of both sites, with the outplant site being much closer to the main fringing reef than the wild thicket (Table 1).

### Fish diversity

Mean fish diversity (Shannon Diversity Index values) varied across sites with different coral substrates (Figure 4;  $F_{3,76} = 6.23$ ,  $P < 0.001$ ). Post hoc comparisons indicated that fish diversity was greater at the outplanted *A. cervicornis* site compared to sites without *A. cervicornis* in Grape Tree Bay ( $P = 0.008$ ) and Preston Bay ( $P = 0.001$ ). Post hoc comparisons indicated fish diversity was not different at the outplanted *A. cervicornis* site compared to the wild *A. cervicornis* site ( $P = 0.064$ ). Fish diversity was greater on average at sites with *A. cervicornis* than sites without *A. cervicornis* ( $t = 3.32$ ,  $P = 0.001$ ,  $df = 76$ ).

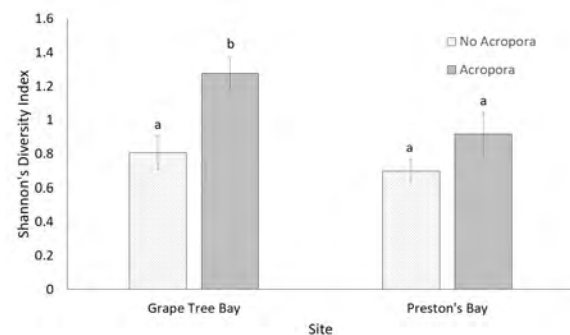


Figure 4: Diversity of fish assemblages at sites with and without *A. cervicornis* cover in two locations. Shannon index value differs significantly among sites (ANOVA,  $F_{3,76} = 6.23$ ,  $P < 0.001$ ,  $n = 20$  fish survey counts per site). Different letters above bars indicate significant differences among sites (determined by post hoc Tukey's HSD test). Values are means  $\pm 1$  SE.

### Fish abundances

There was a significant difference in mean total fish abundance across sites with different coral substrates (Figure 5;  $F_{3,76} = 7.19$ ,  $P < 0.001$ ). Although post hoc comparisons showed that there was no significant difference in fish abundances between sites with wild *A. cervicornis* and without *A. cervicornis* in Preston Bay, there was a trend indicating greater abundances at the patch reef with *A. cervicornis* ( $P = 0.424$ ). Post hoc comparisons also indicated that there was no significant difference in total fish abundance between sites without *A. cervicornis* on either side of the island ( $P = 0.738$ ). Fish abundance was greater at the outplanted *A. cervicornis* site than the wild *A. cervicornis* site ( $P = 0.011$ ). Post hoc comparisons also indicated that fish abundance was greater at the outplanted *A. cervicornis* site than the site without *A. cervicornis* in Grape Tree Bay ( $P = 0.007$ ) and the site without *A. cervicornis* in Preston Bay ( $P < 0.001$ ).

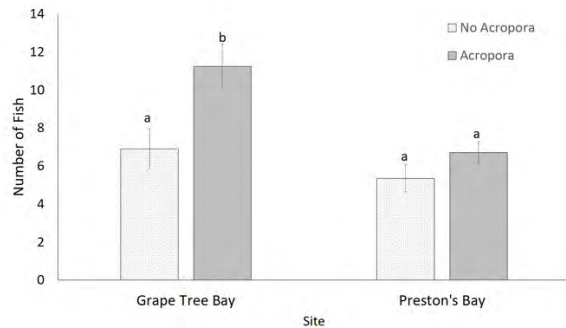


Figure 5: Fish abundance at sites with and without *A. cervicornis* cover in two locations. Average fish abundance differs significantly among sites (ANOVA,  $F_{3,76} = 7.19$ ,  $P < 0.001$ ,  $n = 20$  fish survey counts per site). Different letters above bars indicate significant differences among sites (determined by post hoc Tukey's HSD test).

Parrotfish abundance was different across sites with different coral substrates (Figure 6;  $F_{3,76} = 5.60$ ,  $P = 0.002$ ). Post hoc comparisons indicate that parrotfish abundances were greater at the wild *A. cervicornis* site than sites without *A. cervicornis* in Grape Tree Bay ( $P = 0.026$ ) and Preston Bay ( $P = 0.009$ ). Post hoc comparisons also indicate that parrotfish abundances were greater at the outplanted *A. cervicornis* site than sites without *A. cervicornis* in Grape Tree Bay ( $P = 0.045$ ) and Preston Bay

( $P = 0.026$ ). Parrotfish abundances were not different between wild and outplanted *A. cervicornis* sites ( $P = 0.984$ ).

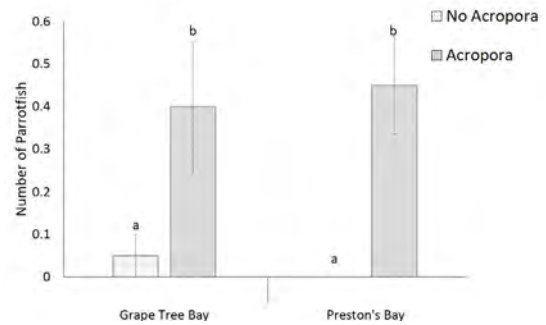


Figure 6: Abundance of parrotfish at sites with and without *A. cervicornis* in two locations. Average parrotfish abundance differs significantly among sites.

Mean Damselfish abundance varied across sites with different coral substrates (Figure 7;  $F_{3,76} = 9.95$ ,  $P < 0.001$ ). Post hoc comparisons indicate that damselfish abundance was greater at the outplanted *A. cervicornis* site compared to the wild *A. cervicornis* site ( $P < 0.001$ ). Post hoc comparisons also indicated that damselfish were more abundant at the outplanted *A. cervicornis* site compared to sites without *A. cervicornis* in Grape Tree Bay ( $P < 0.001$ ) and Preston Bay ( $P < 0.001$ ).

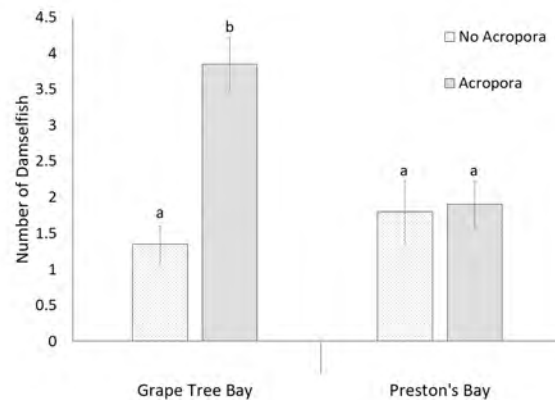


Figure 7: Abundance of damselfish at sites with and without *A. cervicornis* in two locations. Average damselfish abundance differs significantly among sites.

Mean abundance of French grunts was also significantly different across sites with different coral substrates ( $F_{3,76} = 3.76$ ,  $P = 0.014$ ). Post hoc contrasts indicate that French grunt abundance was greater at the outplanted *A. cervicornis* site compared to the site without staghorn coral in Grape Tree Bay ( $P = 0.022$ ).

French grunt abundances were not different between wild and outplanted *A. cervicornis* sites ( $P = 0.051$ ), nor between sites without staghorn corals in Grape Tree Bay and Preston Bay ( $P = 0.988$ ). There was no significant difference in mean abundance of wrasses ( $F_{3,76} = 1.57$ ,  $P = 0.204$ ), blue tangs ( $F_{3,76} = 2.61$ ,  $P = 0.058$ ), mutton snapper ( $F_{3,76} = 0.93$ ,  $P = 0.430$ ), bar jack ( $F_{3,76} = 0.76$ ,  $P = 0.521$ ), or goldline blennies ( $F_{3,76} = 2.11$ ,  $P = 0.106$ ) across sites.

## DISCUSSION

Staghorn coral presence influenced abundance and diversity of fish assemblages. Our results showed that sites with *A. cervicornis* tended to have a higher diversity of fish assemblages when compared to similar sites without *A. cervicornis* cover. *A. cervicornis* provides rigid three-dimensional structural complexity to reefs through both vertical and horizontal growth of hard branches. Increases in structure (rugosity) and greater surface area of hard corals have been correlated with significant increases in fish diversity (Gratwicke and Speight, 2005). Additionally, our results showed that overall fish abundance tended to be greater at sites with *A. cervicornis* than sites without *A. cervicornis*. This finding is consistent with previous work, which has shown a positive relationship between reef complexity and fish abundance (Komyakova et al., 2013). These results support our hypothesis that presence of *A. cervicornis* can cause an increase in fish diversity and abundance and suggest that enhancing reef complexity through the outplanting of staghorn corals may be important to maintain ecosystem diversity and functions.

Our results indicate that two families of fish that are significant to reef health, parrotfish and damselfish, are more abundant where *A. cervicornis* is present, in both the wild and outplanted thickets. Parrotfish have a positive effect on the reef and are crucial for reducing competition between adult corals and macroalgae as they graze exclusively on algae associated with dead coral substrates (Bruggemann 1994), as well as increasing coral recruitment as parrotfish play an important role in preparing dead coral surfaces for coral larval settlement (Bellwood et al. 2004). After the notable decline of Caribbean sea urchin

populations in 1983, parrotfish became the primary grazers of algae (Mumby 2007). Before 1983, sea urchins, along with parrotfish, maintained an equilibrium of high coral cover however, without the sea urchins, reefs were transitioning into a macroalgae dominated systems. Because parrotfish are now the dominant grazers, it is crucial for the success of coral restoration that parrotfish remain abundant, and our study suggests that *A. cervicornis* provide an attractive structure for parrotfish. Based on our field observations, juvenile stoplight parrotfish and princess parrotfish were nearly twice as abundant in stands of staghorn coral compared to nearby corals at patch reefs lacking branching coral. This observation may suggest that *A. cervicornis* corals are an attractive refuge for young parrotfishes, which often hide from reef predators by seeking cover (Graham & Nash 2013).

Damselfish, conversely, are generally considered to have a negative effect on coral reefs, as they promote algal growth. Some species of damselfish exclude herbivorous fish, like parrotfish, from their territory, preventing algal grazing on patches in coral reefs, which in turn promotes an increase in algal biomass and percent-cover (Sammarco 1983). Given that algae directly compete with coral, these territorial behaviors, along with the gardening of algae by some species, significantly suppress coral growth to the point that mortality of coral significantly increases within a few months of damselfish occupation (Potts 1977). Schopmeyer et al. (2015) find that impacts of damselfish algal lawns on *A. cervicornis* colonies caused the highest level of tissue mortality among all coral stressors that they evaluated. Our data suggests that damselfish are also attracted to *A. cervicornis* thickets, both wild and outplanted, though it is unclear whether they have established a territory in the thicket or not. Damselfish abundance has been shown to increase as coral colony size and complexity increases, however, Schopmeyer et. al (2015) suggest that although the negative effects of damselfish can be detrimental to restoration efforts, negative effects can be minimized with strategic coral restoration design. We found that damselfish abundances were significantly greater at the *A. cervicornis* outplanting than all



other patch reefs surveyed. This high abundance, particularly of Yellowtail and Beaugregory damselfish, may be attributed to the fact that the outplanted staghorn thicket is the only patch of such corals in the area, and thus it may be attracting abnormally high densities of the fish if it provides a favorable habitat. Future *A. cervicornis* outplanting efforts around Little Cayman could attempt to establish several thickets at a time as to minimize the effects of damselfish crowding at a limited habitat resource to increase the likelihood of coral survival. Despite the negative effects of some damselfish species, it is important to note that damselfish can occasionally have positive effects on corals since they exclude corallivores, such as types of sea snails (*Coralliophila* sp.) and fireworms (*Hermodice* sp.) (Schopmeyer 2015). Further studies should investigate more closely how the higher abundances of yellowtail, beaugregory, and sergeant major damselfishes we observed impact staghorn thickets.

Further studies could investigate the relationship between density of *A. cervicornis* and composition of reef fish assemblages. Higher coral densities create higher structural complexity, which is usually correlated with increases in fish abundance (Agudo-Adriani et al., 2016). There were a number of factors that we could not control in this study that may have influenced the fish assemblages we observed. For example, the two patch reefs we surveyed in Grape Tree Bay were positioned significantly closer to the main reef crest than the two in Preston Bay, which could have led to lower fish abundances at the latter sites, as there are less available habitats nearby (Menard et al., 2012). It is also possible that the total area of patch reef may affect fish diversity and abundance, as following the tenants of island biogeography theory, larger areas provide more habitat niches. Given the smaller size of the patch reefs surveyed in Preston Bay, the reduced reef size may also help explain the lower abundances and diversity of fishes compared to the northern side of the island. Possible variation in abiotic conditions associated with location of sites, such as tide height, water temperature, or water current velocity could be examined in future studies. Finally, the correlation between damselfish and *A. cervicornis* could be clarified

by determining if damselfish preferentially establish territories in *A. cervicornis* thickets, and if they do, what factors may influence territory choice. Understanding what type of habitat damselfish are attracted to could help improve restoration design and minimize the negative effects of damselfish on coral.

Our study shows that *A. cervicornis* thickets significantly increase diversity and abundance of different fish species, thus functioning as a highly valuable species which should be prioritized in continued outplanting efforts. Our findings also suggest that a recently outplanted thicket of *A. cervicornis* supports greater abundances and diversity of fish species than a comparable patch reef lacking these corals, suggesting that human outplants of the species provide considerable benefits.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

Agudo-Adriani, E. A., Cappelletto, J., Cavada-Blanco, F., & Croquer, A. 2016. Colony geometry and structural complexity of the endangered species *Acropora cervicornis* partly explains the structure of their associated fish assemblage. *PeerJ*, 4: 1861.

Bell, J. D., & Galzin, R. (1984). Influence of live coral cover on coral-reef fish communities. *Marine Ecology Progress Series*, 15: 265–274.

Bellwood, D. R., Hughes, T. P., Folke, C., & Nyström, M. 2004. Confronting the coral reef crisis. *Nature*, 6994, 827–833.

Bohnsack, J. A. & Bannerot, S. P. 1986. A stationary visual census technique for quantitatively assessing community structure of coral reef fishes. NOAA Technical Report NMFS 41: 1-8.

Brock, R. E. 1979. An experimental study on the effects of grazing by parrotfishes and role of refuges in benthic community structure. *Marine Biology*, 4, 381–388.

Bruggemann, J. H., Begeman, J., Bosma, E. M., Verburg, P., & Breeman, A. M. (1994). Foraging by the stoplight parrotfish *Sparisoma viride*. II. Intake and assimilation of food, protein and energy. *Marine Ecology Progress Series*, 57-71.

Graham, N. A. J., Nash, K. L. 2013. The importance of structural complexity in coral reef ecosystems. *Coral Reefs*, 32: 315-326.

Gratwicke, B., & Speight, M. R. 2005. The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. *Journal of Fish Biology*, 3: 650–667.

Hughes, T. P., Kerry, J. T., Álvarez-Noriega, M., Álvarez-Romero, J. G., Anderson, K. D., Baird, A. H., ... Wilson, S. K. 2017. Global warming and recurrent mass bleaching of corals. *Nature*, 7645: 373–377.

Komyakova, V., Munday, P. L., & Jones, G. P. 2013. Relative Importance of Coral Cover, Habitat Complexity and Diversity in Determining the Structure of Reef Fish Communities. *PLOS ONE*, 8: 12.

Ménard, A., Turgeon, K., Roche, D. G., Binning, S. A., & Kramer, D. L. 2012. Shelters and Their Use by Fishes on Fringing Coral Reefs. *PLOS ONE*, 6

Mumby, P. J., Hastings, A., & Edwards, H. J. 2007. Thresholds and the resilience of Caribbean coral reefs. *Nature*, 71: 66- 98.

Potts, D. C. 1977. Suppression of coral populations by filamentous algae within damselfish territories. *Journal of Experimental Marine Biology and Ecology*, 3: 207-216.

Sammarco, P. W. 1983. Effects of fish grazing and damselfish territoriality on coral reef algae. I.

Algal community structure. *Marine Ecology Progress Series*, 1-14.

Schopmeyer, S. A., & Lirman, D. 2015. Occupation dynamics and impacts of damselfish territoriality on recovering populations of the threatened staghorn coral, *Acropora cervicornis*. *Plos one*, 11.

# SUPPLEMENTARY TABLE

Supplementary Table 1: Average number of fish observed at each of the locations, over 20 1-minute bouts. Damselfish, Parrotfish, and other fish are grouped. GN = Grape Tree Bay, No Acropora; GO = Grape Tree Bay, Outplanting; PN = Preston's Bay, No Acropora; PW = Preston's Bay, Acropora

	GN	GO	PN	PW
<b>All</b>				
<b>Damselfish</b>	<b>1.4</b>	<b>3.9</b>	<b>1.8</b>	<b>1.9</b>
Beaugregory	1.2	2.4	1.6	1.8
Yellowtail	0.1	1.1	0.0	0.1
Damselfish				
Sergeant	0.1	0.4	0.2	0.1
Major				
<b>All</b>	<b>0.0</b>	<b>0.4</b>	<b>0.0</b>	<b>0.5</b>
<b>Parrotfish</b>				
Stoplight	0.0	0.4	0.0	0.2
Parrotfish				
Princess	0.0	0.0	0.0	0.2
parrotfish				
Rainbow	0.0	0.1	0.0	0.1
parrotfish				
<b>Other</b>	<b>3.5</b>	<b>3.4</b>	<b>2.2</b>	<b>2.8</b>
Clown	1.9	2.0	1.4	1.2
Wrasse				
Slippery	0.9	0.4	0.3	0.8
Dick				
French	0.2	0.6	0.2	0.2
Grunt				
Blue tang	0.4	0.2	0.0	0.3
Bar jack	0.0	0.1	0.2	0.2
Mutton	0.0	0.1	0.2	0.1
snapper				
Squirrel Fish	0.2	0.0	0.0	0.0
Blue Head	0.0	0.1	0.0	0.1
Wrasse				
Saddled	0.0	0.0	0.1	0.0
Blenny				
Creole	0.0	0.1	0.0	0.0
Wrasse				

## DEGREE OF BLEACHING IN THREE SPECIES OF CARIBBEAN CORALS AND HOW IT MAY INDICATE FUTURE CHANGES IN CORAL REEF COMPOSITION

GRACE S. CALLAHAN AND ANGELA E. ORTLIEB

Teaching Assistants: Michael B. Brown and Debora Goedert  
Faculty Editor: Celia Y. Chen

**Abstract:** When faced with environmental stressors, coral colonies lose crucial endosymbiotic zooxanthellae in a process known as coral bleaching. Many species of coral are unable to meet their daily energetic requirements without zooxanthellae, and colonies can die if they do not recover quickly. Different coral species respond to periods of environmental stress in different ways. Some species are resistant to bleaching events, and therefore endure these events with minimal to no bleaching. Some species, while less resistant to initial bleaching, are more resilient and able to recover after experiencing significant bleaching. The success of these strategies may change as the frequency of bleaching events changes. Corals that show strong resilience, but not necessarily resistance, are often better able to acclimatize to annual bleaching events than resistant corals. To investigate species-specific bleaching resistance in a system that experiences relatively infrequent bleaching events, we estimated coral health through evaluation of colony color in three different species of Caribbean reef-building corals around Little Cayman Island: *Agaricia agaricites* (a plating coral) *Porites astreoides* (an encrusting coral), and *Siderastrea radians* (a small mounding coral). We found that *A. agaricites* is more susceptible to isolated bleaching than *P. astreoides* and found *S. radians* to be the most resistant of all. This suggests that although the bleaching events in Little Cayman are recurring, they are infrequent enough to favor corals with high bleaching resistance over those that can acclimatize to multiple bleaching events. Considering predictions of annual bleaching events globally by 2045 and even sooner in the Caribbean, it is important to study the differential responses of coral species to varying frequencies of stressful environmental events.

### INTRODUCTION

Globally, coral reefs have experienced drastic reductions in size and shifts in species composition over the past several decades (Berkelmans and van Oppen 2006). Coral bleaching, a process driven by extreme environmental conditions (such as prolonged elevated sea temperatures, changes in salinity, erosion, and physical disturbances), involves the loss of crucial endosymbiotic zooxanthellae and results in the pale white appearance of a coral colony (Hoegh-Guldberg 1999). These zooxanthellae can provide up to 90% of a colony's energetic needs; therefore, many species of coral cannot exist in this bleached state for prolonged periods of time (Berkelmans and van Oppen 2006). While it is possible for corals to recover from bleaching events, this recovery is not immediate. Delayed recovery results in a period of increased susceptibility of the affected coral to disease and further bleaching events (Baker et al. 2008). Climate change models predict extreme environmental events to increase in frequency and magnitude over the next century (Fabina et al. 2015).

Considering the vulnerability of coral reefs to extreme environmental events and their critical role in maintaining biodiversity in marine ecosystems, this prediction of increased frequency and magnitude of potential bleaching events raises concern for the future of many marine reef systems.

Studies have begun to investigate the differential responses of species and forms of coral to bleaching events of varying frequencies, because at the current rate of CO<sub>2</sub> emissions, models predict that global bleaching events will occur annually by 2040 and that the Caribbean will experience these events as early as 2025 (van Hooidonk et al. 2013). Research has found that some species, such as the encrusting, reef-building Caribbean coral *Porites astreoides*, show relatively high resistance to isolated bleaching events, but decreased survival after successive annual bleaching events (Grottoli et al. 2014). One explanation for this decrease in resistance after repeated bleachings is that, like most defensive strategies, bleaching tolerance is costly (Mole 1994). Therefore, bleaching resistance and bleaching resilience could

represent a trade-off, such that a species that allocates substantial energy to resistance might demonstrate diminished resilience to recurring bleachings (Grottoli et al. 2014). However, there are some species that have been documented to show both high resistance and high resilience, such as the small mounding coral *Siderastrea radians* (Lirman and Manzello 2008). However, this resistance and resilience is in response to high salinity and sedimentation levels; it is unknown whether this can translate to tolerance of sustained periods of increased temperatures.

In 2009, a severe isolated bleaching event affected coral reefs throughout Little Cayman Island (van Hooidonk et al. 2012). Immediately following this 2009 bleaching event, researchers at the Central Caribbean Marine Institute (CCMI) evaluated coral health on reefs around the island and found that *P. astreoides* was more resistant to bleaching than *Agaricia agaricites* (van Hooidonk et al. 2012). Since 2009, there have been at least two bleaching events in reefs around Little Cayman. In 2015, reefs around the world experienced extensive bleaching due to elevated ocean temperatures brought by El Niño, marking the longest global mass coral die-off in recorded history (Eakin et al. 2016). According to researchers at CCMI, the next bleaching event to affect the reefs around Little Cayman was in September 2017 (Correia K., *pers. comm.*). Bleaching events around Little Cayman have increased in frequency over the past decade and now appear to be about two years apart.

While the frequency of bleaching events in Little Cayman has increased, these events have not yet become annual. We hypothesize that this increase in frequency is not yet drastic enough for the costs of bleaching resistance to outweigh the benefits; therefore, coral species that are initially resistant should be in better health than species that lack strong resistance but may show higher resilience and ability to acclimatize to annual bleaching events. Based on the resistance of both *P. astreoides* and *A. agaricites* documented after the isolated bleaching event of 2009, and the tolerance to bleaching displayed by *S. radians* in response to other environmental stressors (Lirman and Manzello 2008), we predict that *S. radians* will show lower degrees of bleaching than both *P. astreoides* and *A. agaricites* and that *P. astreoides* will show lower

degrees of bleaching than *A. agaricites* six months following the 2017 bleaching event. Alternatively, it is possible that bleaching events do not need to be annual for the costs of bleaching resistance to outweigh the benefits, resulting in a decline in health since 2009 of those corals known to be most resistant. If this is the case, we would see *P. astreoides* and *S. radians* displaying higher degrees of bleaching than *A. agaricites*.

## METHODS

To assess the health of different Caribbean reef-building corals around Little Cayman Island, we sampled coral colonies along reefs in Grape Tree Bay and Preston Bay on the North and South sides of the island, respectively. At each site, we selected five 10-meter transects and counted all coral colonies that fell directly beneath each transect. We recorded species, depth, maximum length, width, and height of each colony. Using a Coral Watch Coral Health Chart (Fig. 1), we recorded the darkest and lightest color on each colony and the numerical values that corresponded to these colors. We quantified the difference between these numerical values, which is an estimate for bleaching severity and inversely related to coral health. Differences that are above two color values represent significant loss of zooxanthellae and chlorophyll a content, and therefore indicate bleaching (Siebeck et al. 2006). We encountered 11 species of coral along the transects but restricted our analyses to only the most abundant three: *Porites astreoides*, *Agaricia agaricites*, and *Siderastrea radians*. A table listing all of the coral species that we found, their abundance and their severity of bleaching can be found in the supplementary material (Supplementary Table 1).

## Statistical analyses

We used a two-way ANOVA with an interaction term to determine the effects of species and site on observed differences in degrees of bleaching and used a post-hoc t-test to analyze the relationships between each focal species within a single location.



Figure 1. The chart used to evaluate bleaching severity of a coral colony using the lightest and darkest colors present on a single colony. Each side of the chart represents a common coral shade with decreasing values corresponding to increasingly severe bleaching.

## RESULTS

At both sampling locations, *A. agaricites* exhibited the highest degree of bleaching, followed by *P. astreoides*, and then *S. radians*. At Grape Tree Bay, the average bleaching severity values for *P. astreoides*, *A. agaricites*, and *S. radians* were 3.1, 2, and 1.5, respectively. At Preston Bay, the average bleaching severity values were 2.6, 2, and 1.3 (Fig. 2). A value of 2 or more indicates bleaching. Degrees of bleaching were significantly different between species ( $F_{2,28} = 6.32$ ,  $P < 0.01$ ) while site had no influence on degree of bleaching ( $F_{1,28} = 0.57$ ,  $P = 0.46$ ) and there was no interaction between species and site ( $F_{2,28} = 0.24$ ,  $P = 0.79$ ). At Grape Tree Bay, the degree of bleaching of *A. agaricites* was significantly greater than both *P. astreoides* and *S. radians* ( $t = 2.31$ ,  $P < 0.03$ ,  $DF = 28$ ). There was no difference in degree of bleaching between *P. astreoides* and *S. radians*. At Preston Bay, the degree of bleaching of *A. agaricites* was significantly greater than only *S. radians* ( $t = 2.67$ ,  $P = 0.01$ ,  $DF = 28$ ). Although there was no significant difference in degree of bleaching between *A. agaricites* and *P. astreoides* at Preston Bay, we see the same trend of relative bleaching severity values at both sites.

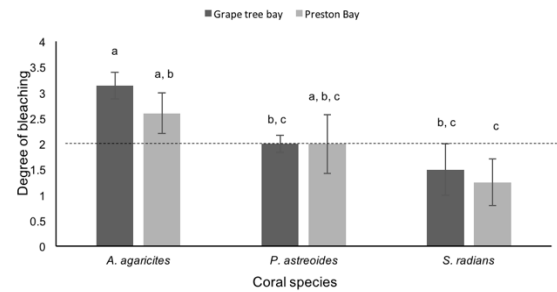


Figure 2. Degree of bleaching (the mean difference between the darkest color value and the lightest color value found on a coral colony using the Coral Watch Coral Health Chart) of three species of coral at two sites. Letters denote significant relationships. Degrees of bleaching above two (dashed line on figure) indicate significant bleaching.

## DISCUSSION

We found that bleaching severity differs between coral species in the Caribbean reef system around Little Cayman, indicating varying species responses to bleaching events. Using the estimated degree of bleaching as a proxy for health, we found that *P. astreoides* was healthier than *A. agaricites* (Fig. 2), which matches bleaching resistance documented after the 2009 bleaching event (van Hooidonk et al. 2012). Despite the slight increase in frequency of bleaching events prior to 2018, our data show that *P. astreoides* is still in better health than *A. agaricites*, leaving us to conclude that the increase in frequency of bleaching events has not yet changed the patterns of relative coral health in this area.

We hope that future studies will address not only bleaching severity, but the densities of coral species and how these change over time. Varying resiliencies and abilities to acclimatize to changing water temperatures will alter the coral composition of reefs, and in all probability this alteration will come in the form of a reduction of coral biodiversity. Many coral reefs in the Caribbean have shown an increase in relative density of *P. astreoides* over the last 30 years, even as the percent of total coral cover has declined (Green et al. 2008). Coral species that are often the most at risk are those that are branching and structurally complex, such as *Acropora*. The replacement of these branching, reef-building species by smaller, more rapidly growing species such as *P. astreoides* (Green et

al. 2008) will result in reefs that are less topographically complex. Many marine organisms rely on large branching corals for suitable habitats, and these reductions will make survival much more difficult for these individuals.

Given that we found *P. astreoides* colonies in good health, it seems likely that reefs around Little Cayman will experience a shift in composition that favors this species over more complex, branching corals. However, the transition to a reef dominated by *P. astreoides* is contingent on the fact that bleaching events are not annual, because annual bleaching has been shown to decrease the health of *P. astreoides* (Grottoli et al. 2014). If bleaching events in the Caribbean do indeed occur annually by 2025 (Grottoli et al. 2014, van Hooidonk et al. 2013), we can predict that reefs might experience another dramatic change in composition, where initially resistant species such as *P. astreoides* may decrease in density while species that are better able to acclimatize may increase in density. These hypothesized changes will affect not only coral, but all species that are dependent on reef ecosystems. Understanding how reef biodiversity may respond to changing environmental conditions may help inform and focus long-term marine conservation research and policy.

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#### AUTHOR CONTRIBUTIONS

Both authors contributed equally.

#### LITERATURE CITED

- Baker, A. C., P. W. Glynn, B. Riegl. 2008. Climate change and coral reef bleaching: An ecological assessment of long-term impacts, recovery trends and future outlook. *Estuarine, Coastal and Shelf Science* 80:435-471.
- Berkelmans, R. and J. H. van Oppen. 2006. The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences* 273: 2305–2312.
- Eakin, C. M., G. Liu, A. M. Gomez, J. L. De La Cour, S. F. Heron, W. J. Skirving, E. F. Geiger, K. V. Tirak, and A. E. Strong. 2016. Global coral bleaching 2014-2017. *The News Journal of the International Society for Reef Studies Reef Currents* 31(1): 20-26.
- Fabina, N. S., M. L. Baskett, and K. Gross. The differential effects of increasing frequency and magnitude of extreme events on coral populations. *Ecological Applications* 25: 1534-1545.
- Green, D., Edmunds, P., Carpenter, R.C., 2008. Increasing relative abundance of *Porites astreoides* on Caribbean reefs mediated by an overall decline in coral cover. *Marine Ecology Progress Series* 359: 1-10.
- Grottoli, A. G., M. E. Warner, S. J. Levas, M. D. Aschaffenburg, V. Schoepf, M. McGinley, J. Baumann, Y. Matsui. 2014. The cumulative impact of annual coral bleaching can turn some coral species winners into losers. *Global Change Biology* 20: 3823-3833.
- Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Marine and Freshwater Research* 50: 839-866.
- Lirman, D. and D. Manzello. 2008. Patterns of resistance and resilience of the stress-tolerant coral *Siderastrea radians* (Pallas) to sub-optimal salinity and sediment burial. *Journal of Experimental Marine Biology and Ecology* 369: 72-77.
- Mole, S. 1994. Trade-offs and constraints in plant-herbivore defense theory: a life-history perspective. *Oikos* 71(1): 3-12.
- van Hooidonk, R. J., D. P. Manzello, J. Moye, M. E. Brandt, J. C. Hendee, C. McCoy, and C. Manfrino. 2012. Coral bleaching at Little Cayman, Cayman Islands 2009. *Estuarine, Coastal, and Shelf Science* 106: 80-84.



## SUPPLEMENTARY TABLE

Supplementary Table 1. All coral species found during experiment with corresponding sites, health values (calculated using Coral Watch Coral Health Chart), and total number of colonies. Averages were calculated in all instances with more than one colony. "Fluorescing" indicates *P. porites* that has turned purple.

Species	Site	Average Coral Health	n
<i>Agaricia agaricites</i>	Grape Tree Bay	3.1	1
	Preston Bay	2.6	1
<i>Acropora cervicornis</i>	Grape Tree Bay	2.0	1
<i>Dichocoenia stokesi</i>	Grape Tree Bay	3.0	6
	Preston Bay	0.0	2
<i>Orbicella annularis</i>	Grape Tree Bay	1.5	1
<i>Orbicella faveolata</i>	Grape Tree Bay	2.0	9
	Preston Bay	0.0	3
<i>Porites astreoides</i>	Grape Tree Bay	2.0	1
	Preston Bay	2.0	2
<i>Porites furcata</i>	Grape Tree Bay	2.0	1
	Preston Bay	2.0	7
<i>Porites porites</i>	Grape Tree Bay	fluorescing	6
	Preston Bay	2.3	5
<i>Pseudodiploria strigosa</i>	Grape Tree Bay	2.0	5
	Preston Bay	0.0	1
<i>Siderastrea radians</i>	Grape Tree Bay	1.5	2
	Preston Bay	1.3	8
<i>Siderastrea siderea</i>	Grape Tree Bay	1.5	4

## PATTERNS IN THE DISTRIBUTION OF ENCRUSTING SPONGE ACROSS REEF ZONES

HANNAH I. HOFFMAN, MANAAV JALAN, AND PAUL J. VICKERS

Teaching Assistants: Debora Goedert and Michael B. Brown

Faculty Editor: Celia Chen

**Abstract:** Sponges play a critical role in Caribbean reefs by cycling nutrients, interacting with other inhabitants such as macroalgae, and colonizing reefs with dead coral. However, their distribution and ecology are little understood in most coral reef ecosystems. We examined sponge colony density, coverage, and substrate use of an orange encrusting sponge in Grape Tree Bay, Little Cayman across patch and fringing reef zones. Within 10 five-meter transects randomly placed in each reef zone, we found that sponge colony density did not vary based on reef zone, but the sponge covered a larger area in the fringing reef zone. Additionally, we found sponge on only *Briareum asbestinum* and remnant coral substrate. There was the same amount of *B. asbestinum* substrate in both reef zones but more remnant coral substrate in the fringing reef. In the fringing reef zone, sponge colonies were denser and covered more area on remnant coral than on *B. asbestinum*. These results suggest that fringing reefs may host more sponge area due to higher availability of remnant coral substrate.

**Key words:** *Briareum asbestinum*, coral reefs, encrusting sponge, substrate preference

### INTRODUCTION

Coral reefs are one of the most productive ecosystems on the planet, supporting large and diverse communities of animals. However, coral coverage in reefs has recently fallen, with an 80 percent decline in only three decades (Gardner et al. 2003). This reduction has marked an environmental shift, with an increasingly large portion of reef space occupied by organisms such as sponges and macroalgae (Aronson et al. 2002; González-Rivero et al. 2011).

While the competitive interaction between coral and macroalgae has been well-documented, sponges have recently emerged as important players in reefs, influencing the eventual stable state that these ecosystems reach (González-Rivero et al. 2011). For example, sponges may reduce herbivory pressure on algae by providing an alternative food source, helping algae outcompete corals and shifting reefs to a sponge-algae regime (González-Rivero et al. 2011). Additionally, coral recruitment could decline in reefs dominated by sponges, as coral larvae cannot successfully settle on sponge covered substrate (Goldberg 2013). Contrarily, sponges compete for space with corals less aggressively than macroalgae. If sponges outcompete algae for space on dead coral, an increase in sponge abundance may actually providing a more favorable environment for the surviving coral than one with algal dominance

(Marulanda-Gómez et al. 2017). Additionally, sponges provide crucial ecosystem services such as nutrient cycling that benefit the reef ecosystem as a whole (Bell 2008, de Goeij et al. 2013). Given this wide range of functionality, it is valuable to better understand sponge ecology, particularly in reefs that face declining coral health.

One characteristic that could inform the role of sponges in marine ecosystems is their density and coverage within reefs. Sponges disperse through a variety of sexual and asexual reproductive strategies to increase abundance and expand into new territory. While sexual reproduction tends to be unpredictable and ineffectual in areas not yet established by sponges, these organisms often use asexual modes such as budding and fragmentation (Ayling 1980; Goldberg 2013). Such methods of reproduction allow sponges to disperse between reef zones and within continuous sections of reef. Habitat may also play a role in sponge dispersal by influencing fragmentation, substrate for settlement, and growth rates. Previous research has shown differences in sponge growth rates on specific substrates between species (Plucer-Rosario 1986; Mclean et al. 2015). Some sponges grow best on remnant coral (Plucer-Rosario 1986) while others favor octocorals (Mclean et al. 2015). Fragmentation rates also vary based on reef zone, with sponge

fragmentation increasing with higher mechanical energy or wave action (Ruppert et al. 2004).

To evaluate the effect of habitat on sponge colony density and coverage, we conducted a survey of a common species of encrusting sponge in the back reef of Grape Tree Bay, Little Cayman (Figure 1). We evaluated differences in sponge colonization between two reef zones – patch reef (PR) and fringing reef (FR) – and among the substrates within these zones. We hypothesized that sponge abundance would be higher in FR due to its contiguous nature, which provides easily-reachable settlement areas for new sponge fragments. The sponge colonies may also be older and larger in the FR due to a higher chance of original colonization as a result of this reef's direct exposure to ocean currents. Additionally, sponges may grow disproportionately on certain reef substrate types; for instance, previous literature suggests that some sponges colonize dead coral more effectively than live coral (Plucer-Rosario 1986). Sponge abundance could differ between reef zones due to potential substrate composition differences between patch and fringing reef.

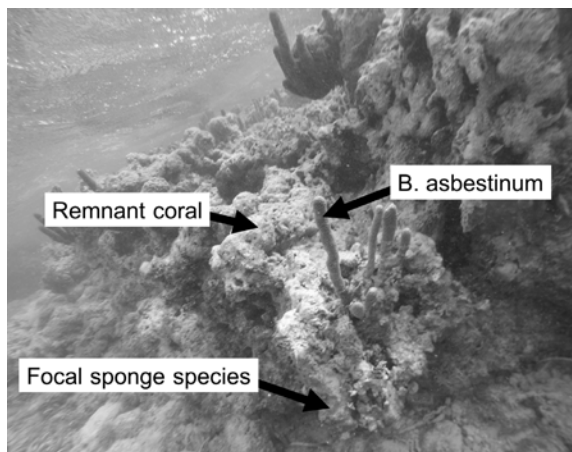


Figure 1. Focal sponge species growing on remnant coral and *Briareum asbestinum*.

#### METHODS

We conducted our study in Grape Tree Bay, Little Cayman from February 21 to February 23, 2018. For both patch and fringing reef zones, we placed 10 five-meter transects along the sides of reefs. We defined PR as isolated coral mounds located at least 10 meters away from the continuous fringing reef. Because reef height

was relatively constant within a transect, we obtained transect area by multiplying reef height by the five-meter length of the transect. For each sponge colony in the transect, we measured the length and width of each to estimate its area and noted the substrate on which it grew. We classified substrate type into four categories: remnant coral, *B. asbestinum*, live coral other than *B. asbestinum*, and macroalgae. We marked *B. asbestinum* as a unique substrate due to our preliminary observation that the sponge grew on this coral species but not others. We calculated sponge colony density for each transect by dividing the number of sponge colonies by the transect area; and subsequently conducted a Welch's two sample t-test to compare sponge colony density between the patch and fringing reef zones. To evaluate whether sponges covered different amounts of area in patch and fringing reef zones, we ran a Welch's two sample t-test comparing the total sponge area per transect area between the zones. This test was one-tailed given our prediction that sponges would cover more area in FR than in patches due to the fact that they likely colonize FR before spreading to PR. We normalized sponge cover values with a  $\text{Log}(1+x)$  transformation to account for transects without sponge (zero values). These analyses were conducted in R v3.4.1 (R Development Core Team, 2013).

To characterize available substrate in the transects, we positioned 4 quarter-meter quadrats at one-meter intervals starting at the one-meter mark. We placed the first quadrat at the top of the reef wall and moved subsequent quadrats downwards by a quarter meter. On reef sections shorter than one meter, we placed the final quadrat in the middle of the reef wall. We estimated the proportion of the quadrat area covered by each substrate type and then averaged the proportions of each substrate across the four quadrats. Because we categorized reef surface into four substrates, we ran a principal components analysis on the substrate proportions to describe transect composition with one value. Increasing values along the first principal component axis indicated increase in remnant coral and *B. asbestinum* substrate, which were the two substrates on which we found the sponge (Table 1). Decreasing values along the first principal component axis

indicated an increase in live coral and macroalgae substrate, on which we did not find the sponge (Table 1). We compared substrate composition of the two reef zones by running a t-test on values derived from PC-1 loadings. Although PC-1 represented only 47% of the total variance, we used only this axis because PC-2 was difficult to interpret biologically. This principal component analysis was conducted in JMP Pro 13 (SAS Institute Inc. 2007).

Table 1. Loading results of a principal components analysis on available reef substrate types.

Substrate	PC-1	PC-2
<i>B. asbestinum</i>	0.37	0.57
Remnant coral	0.62	-0.34
Live coral	-0.44	0.50
Macroalgae	0.54	-0.44
Variance explained (%)	46.55	32.19

To investigate the possible interaction between reef zone and substrate type on sponge colony density, we ran a linear mixed-effect model with density as the predicted value, reef zone and substrate type as fixed effects, and the transect as a random effect. We ran a similar model to predict sponge cover, with reef zone and substrate type as fixed effects and the transect as a random effect. Sponge cover values were square root transformed to meet the assumption of normality. These analyses used the package “lmerTest” (v. 2.0-33; Kuznetsova et al. 2016) and were conducted in R v3.4.1 (R Development Core Team, 2013).

## RESULTS

We found 76 sponges in 116 square meters of reef (mean  $\pm$  SE =  $0.67 \pm 0.07$  per square meter), which covered an average of 4.5% of the reef wall (mean  $\pm$  SE =  $4.54 \pm 0.79$  %). Sponge colony density did not vary between patch and fringing reef zones ( $t = 0.55$ ,  $p = 0.59$ ,  $df = 15.19$ ) (Figure 2a). However, sponges covered more area in the FR zone than in the PR zone (one-tailed  $t = 1.94$ ,  $p = 0.04$ ,  $df = 13.23$ ) (Figure 2b). In both patch and fringing reef zones, we found sponges only on remnant coral and *B. asbestinum*. Given that PC-1 correlates positively with inhabited sponge substrates and negatively with uninhabited substrates (Table 1),

we found that PR had significantly less suitable habitat than fringing reef (Figure 3;  $t = 3.72$ ,  $P = 0.0017$ ,  $df = 17.26$ ).

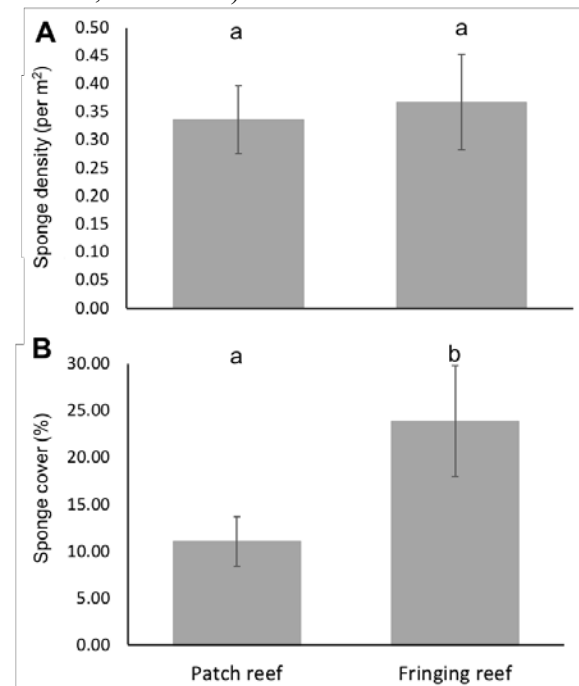


Figure 2. Sponge colony density (A) and cover (B) based on reef zone. Different letters denote significant differences between reef zones.

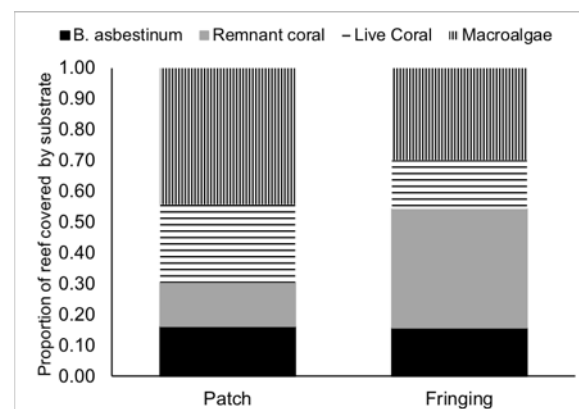


Figure 3. Reef composition based on substrate type in two reef zones.

There were significant interactions between reef zone and substrate type for both sponge colony density and cover. Sponges were least dense and covered the least area on *B. asbestinum* in the FR (Figure 4). Compared to sponges on *B. asbestinum* in the FR, sponges were more dense (Figure 4a;  $t = 3.06$ ,  $p < 0.01$ ,  $df = 34$ ) and covered more area (Figure 4b;  $t = 3.37$ ,  $p < 0.01$ ,  $df = 36$ ) in PR on both substrates,

but colony density and cover did not differ in PR based on substrate (Figure 4a;  $t = 0.92$ ,  $p > 0.05$ ,  $df = 34$ ; Figure 4b;  $t = 0.65$ ,  $p > 0.05$ ,  $df = 36$ ). Sponges were densest (Figure 4a;  $t = 3.90$ ,  $p < 0.001$ ,  $df = 34$ ) and covered the most area (Figure 4b;  $t = 3.94$ ,  $p < 0.001$ ,  $df = 36$ ) on remnant coral in the FR.

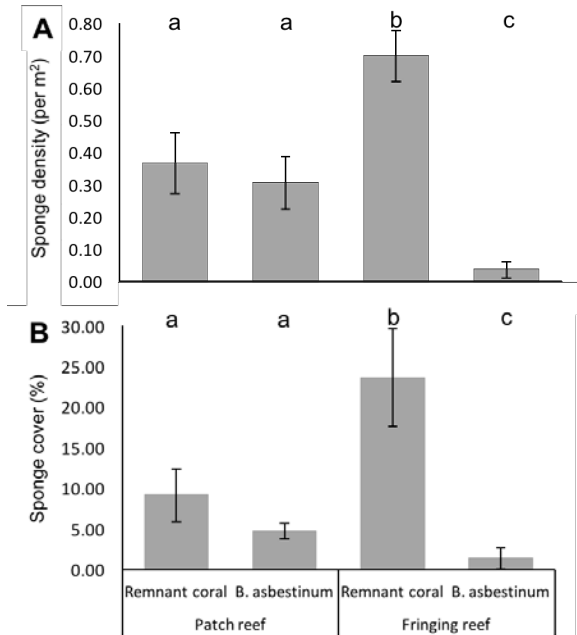


Figure 4. Sponge colony density (A) and coverage (B) based on substrate types in two reef zones. Different letters denote significant differences between substrate types.

## DISCUSSION

We found an average sponge colony density of 0.67 encrusting sponge colonies per square meter in Grape Tree Bay, with comparable density in the two reef zones, patch and fringing reef. However, the sponge covered significantly more area of FR than PR. Sponge distribution correlated with substrate type, as we found that sponges grew only on remnant coral and live *B. asbestinum*; furthermore, the availability of these substrates varied between reef zones, and sponges colonized the substrate types differently depending on the zone.

Considering the diversity of taxa on a coral reef, the consistent presence of one sponge species in our transects warrants further study of this phylum in reef ecosystems. The similarity in densities between patch and fringing reefs suggests no difference in the dispersal of sponge

propagules between these zones. This finding could result from a functional similarity between the contiguous FR and patches, as they are separated by a relatively short distance. Sponge fragments may be able to travel this distance facily given the water current. Even though there was no difference in sponge colony density between reef zones, sponges covered a larger area of the FR, indicating that sponge colonies were larger in the FR. As we hypothesized, this finding could result from original colonization of the FR and thus older sponge age in this zone. In time, fragments from the FR may have traveled to nearby PR.

Substrate compositions may also explain the reefs' differing sponge coverage. We found that there was a greater proportion of macroalgae and live coral (substrates that sponges do not seem to grow on) in the PR and a corresponding greater proportion of remnant coral (a suitable sponge substrate) in the FR. The greater availability of suitable substrate in the FR may also have led to the greater coverage of this zone. The finding that sponges grew larger in the FR could result from the greater availability of remnant coral. Sponges that did not settle on remnant coral inhabited *B. asbestinum*, with a higher recruitment and growth on this substrate in the PR. This trend could be explained by unequal availability of remnant coral in the two reef zones. The proportion of *B. asbestinum* was comparable in both reef zones, but there was a lower availability of remnant coral in the PR zone.

The association between the encrusting sponge and *B. asbestinum* is of particular interest. This soft coral may have lower defenses against sponge encrustation compared to other coral species given that it was the only one inhabited. However, the specific relationship between the two organisms remains ambiguous. McLean et al. (2015) show the existence of a range of antagonistic and mutualistic relationships between sponge and octocoral, such as *B. asbestinum*. It may be antagonistic given that the sponge appears to engulf the coral, blocking polyps and possibly preventing photosynthesis and food capture. Contrarily, McLean and Yoshioka (2008) suggest the possibility of a mutualistic relationship between the two organisms, as some encrusting sponge

species provide structural integrity and anti-herbivory chemical defenses to the coral. Future studies could resolve this relationship.

This study demonstrates that coral reefs are not homogenous habitats, with their structure and surface composition impacting the growth patterns of occupant organisms. Our findings encourage further investigation into the relationship between sponge and macroalgae, which compete for reef space but contribute differently to coral ecosystems. Research could also evaluate the changing composition of substrate availability over time to predict future presence of sponge, algae, and coral in reef environments. Such evaluations could serve to illuminate the impact of the shifting reef regime and aid conservation efforts in predicting resilient areas of coral and at-risk zones.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

- Aronson, R.B., W. Precht, M. Toscano, and K.H. Koltes. 2002. The 1998 bleaching event and its aftermath on a coral reef in Belize. *Marine Biology* 141: 435-447.
- Ayling, A.L. 1980. Patterns of sexuality, asexual reproduction and recruitment in some subtidal marine Demospongiae. *The Biological Bulletin* 158:271-282.
- Aronson, R.B., W. Precht, M. Toscano, and K.H. Koltes. 2002. The 1998 bleaching event and its aftermath on a coral reef in Belize. *Marine Biology* 141: 435-447.
- Bell, J.J. 2008. The functional roles of marine sponges. *Estuarine, Coastal and Shelf Science* 79:341-353.
- de Goeij, J.M., D. van Oevelen, M.J.A. Vermeij, R. Osinga, J. J. Middelburg, A.F.P.M. de Goeij, W. Admiraal. 2013. Surviving in a marine desert: the sponge loop retains resources within coral reefs. *Science* 342: 108-110.
- JMP, Pro 13. SAS Institute Inc., Cary, NC, 1989-2007.
- Gardner, T. A., Côté, I. M., Gill, J. A., Grant A., Watkinson A. R. 2003. Long-term region-wide declines in Caribbean corals. *Science* 301(5635): 958-960.
- Goldberg, W.M. 2013. Reef Sponges. Pages 79-101 in *The Biology of Reefs and Reef Organisms*. The University of Chicago Press: Chicago.
- González-Rivero, M., L. Yakob, and P.J. Mumby. 2011. The role of sponge competition on coral reef alternative steady states. *Ecological Modelling* 222:1847-1853.
- Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2016. lmerTest: Tests in Linear Mixed Effects Models. Retrieved from: <https://CRAN.R-project.org/package=lmerTest>.
- Marulanda-Gómez, Á., M. López-Victoria, and S. Zea. 2017. Current status of coral takeover by an encrusting excavating sponge in a Caribbean reef. *Marine Ecology* 38: e12379.
- McLean, E. L. and P.M. Yoshioka. 2008. Substratum effects on the growth and survivorship of the sponge *Desmapsamma anchorata*. *Caribbean Journal of Science* 44:83-89.
- Mclean, E.L., K. Rützler and P.S. Pooler. 2015. Competing for space: factors that lead to sponge overgrowth when interacting with octocoral. *Open Journal of Marine Science* 5:64-80.
- Plucer-Rosario, G. 1986. The effect of substratum on the growth of *Terpios*, an encrusting sponge which kills corals. *Coral Reefs* 5:197-200.
- R Development Core. 2013. R: A Language and Environment for statistical computing. Vienna Austria: R Foundation for Statistical Computing.
- Ruppert E.E., Fox R.S., Barnes R.D. 2004. Pages 76-90 in *Invertebrate Zoology: A Functional Evolutionary Approach*. Brooks/Cole, 7th ed.



## WHEN THE SURGE HITS THE FAN: SEA FAN (GENUS *GORGONIA*) ORIENTATION IN HYDRODYNAMICALLY COMPLEX SHALLOW REEFS

CLAYTON E. JACQUES, MARY M. PEDICINI, CORINNE R. VIETORISZ, BALTHASAR L. VON HOYNINGEN HUENE

Teaching Assistants: Michael B. Brown and Debora Goedert  
Faculty Editor: Celia Y. Chen

**Abstract:** Body orientation influences feeding success and structural stability in sessile organisms, such as sea fans of the coral genus *Gorgonia*. Past studies have shown that these sea fans tend to orient with their greatest surface area perpendicular to dominant currents; however, multi-directional water flow – as in the case of interactions between current and surge in shallow waters – could complicate fan orientation relative to water movement. In a shallow reef environment, we measured the directions of underwater surge and current, as well as the height, width and planar orientation of the fans of two *Gorgonia* species. Current and surge flowed in different directions, but fan orientation was not perpendicular relative to surge or current. Larger fans did not orient more perpendicularly to the surge. How corals orient spatially could influence reef structures amidst complex water flow.

**Key words:** current, *Gorgonia*, orientation, sea fan, surge

### INTRODUCTION

Sessile organisms face unique challenges to grow and orient without the ability to move freely. Adult corals (class Anthozoa) affix to substrates and form colonies of polyps. Among sea fans (genus *Gorgonia*), which form flat planes, the planar orientation of the sessile colonies greatly impacts exposure to moving seawater. In fact, previous studies indicate that they tend to orient perpendicular to the prevailing current (Wainwright and Dillon 1969, Grigg 1972).

Perpendicular orientation to water movement may improve both structural stability and foraging efficiency of *Gorgonia*. Wainwright and Dillon (1969) found that the alignment of fans' axial centers changes as they grow larger, and the orientation towards perpendicularity seems to occur passively under pressure from the flow of water. With a perpendicular orientation to the current, fans have a stable position with minimal torsion on the fan's structure. A sessile planar organism oriented any way other than directly parallel or perpendicular to a prevailing current would face increased likelihood of fracture (Wainwright and Dillon 1969). Perpendicularity also increases surface exposure to moving water, improving the likelihood of encountering planktonic food items (Grigg 1972). While fan shape could impact hydrodynamic properties, *Gorgonia*

generally exhibit similar growth patterns, more vertical than horizontal.

Variation in water flow direction may confound reorientation behavior, independent of the proposed mechanisms. *Gorgonia* growing in deep waters orient against comparatively consistent current strength and direction (Grigg 1972). However, the physiognomy of shallow coral reefs complicates water movement patterns. The prevailing current directs water movement for the entire area with consistent direction and strength, while waves breaking against reef structures generate brief, regular, and intense surge, often in different directions at different locations. Both types of water movement could exert directional forces on the orientation of the fans.

In our study, we evaluated sea fan orientation relative to both prevailing current and local surge. We also investigated the effect of sea fan surface area on orientation relative to water flow. We tested the hypothesis that sea fans orient perpendicular to the surge direction, a stronger although less consistent force than the current. Moreover, since fan area increases with age, *Gorgonia* with greater surface area have had more time to reorient their growth and therefore may be more closely oriented to the direction of water movement.

## METHODS

### *Orientation of current and surge*

We conducted our study in Grape Tree Bay, at the back reef of the Central Caribbean Marine Institute (CCMI) station on Little Cayman. To determine the direction of the prevailing current, we tracked water flow with marking tape attached to a pole and measured the azimuth of the current with a compass at 5 points spanning our sampling area with a compass (estimated error of 5-10°). Because current direction forms a linear vector, we measured the azimuth of the current between 0 and 180°. We sampled the current direction at roughly equal distance between the shore and the reef to ensure that our measurements represented the dominant current, and not local wave activity. We calculated the mean of these directions to approximate the general current direction.

We followed the same procedure to determine the surge direction at the locations of individual sea fans, taking one measurement at each fan. To test whether surge direction varied from current direction, we calculated the difference in the azimuth between each surge direction and the mean current direction. We used a Wilcoxon Signed-Rank test to compare the distribution against a mean difference of zero.

### *Orientation and area of sea fans*

We identified 55 sea fans of the genus *Gorgonia* in the back reef of the CCMI station. The common sea fan (*G. ventalina*) and Venus sea fan (*G. flabellum*) are not reliably distinguishable in the field, and our study sample likely contained representatives of both species. To determine the orientation of each sea fan, we calculated the azimuth of the plane of the sea fan's surface with a compass (0-180°, estimated error of 5-10°). With a meter stick, we measured the height of the corals from the base of the blade to the top at the longest point, and width at the widest point along the blade.

To determine if the sea fans were oriented perpendicular to the surge or the current, we calculated the absolute value of the difference in the azimuth between the fan's surface and both the surge direction for each fan and the direction of the prevailing current. We examined histograms of the frequency distribution of these

differences and used two one-sample t-tests to compare the mean of the difference against 90° (perpendicular).

To estimate the area of each fan, we multiplied its height measurement by the width. We used a natural log transformation on area measurements in order to normalize the distribution. To create a measure of perpendicularity, we calculated the difference from 90° of the angle between the fan and the surge. We used a linear regression to evaluate the relationship between the transformed area of the fan and the difference from 90° of the fan-surge angle.

## RESULTS

The underlying ocean current in the area sampled had a mean azimuth of 102° (SD =  $\pm 8.37$ ), while the azimuth of water surges ranged between the entire possible angular range of 0° and 180° (mean  $\pm$  SD = 109.5°  $\pm$  65.2°). Surge moved at a different angle than the current (Wilcoxon Signed-Rank,  $t = 742.5$ ,  $P < 0.001$ ,  $df = 53$ ). Sea fans did not orient perpendicular to the surge ( $t = -2.69$ ,  $P = 0.010$ ,  $df = 53$ ) but converged on an orientation of about 75° from the angle of the surge (mean  $\pm$  SD = 76.6°  $\pm$  36.6°) following a normal distribution (Figure 1). The fans did not converge on any specific orientation to the current and the differences between the fan angles and current angle did not follow a normal distribution (Figure 2; mean  $\pm$  SD = 33.6°  $\pm$  22.2°).

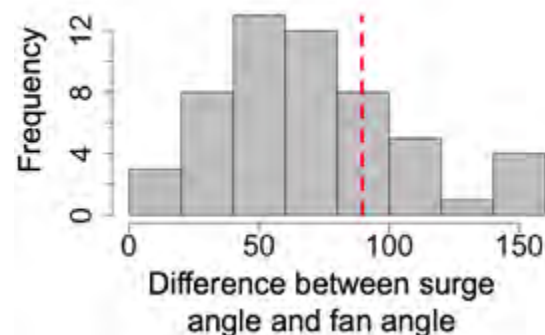


Figure 1. Distribution of the differences between surge angle and fan angle. Dashed red line indicates the expected angle difference (90 degrees) if fans were oriented perpendicular to the surge (N = 54).

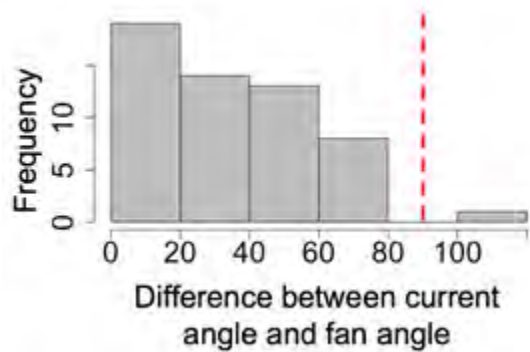


Figure 2. Distribution of the differences between current angle and fan angle. Dashed red line indicates the expected angle difference (90 degrees) if fans were oriented perpendicular to the prevailing current (N = 54).

*Gorgonia* areas ranged between 192 and 4400 cm<sup>2</sup> (mean  $\pm$  SD = 1518.7  $\pm$  1111.4 cm<sup>2</sup>). We found no relationship between natural log-transformed area and perpendicularity of fan orientation (linear regression, slope  $\pm$  SE = -6.87  $\pm$  3.90, P = 0.08, r<sup>2</sup> = 0.06).

#### DISCUSSION

We determined that the direction of current and surge were different. Thus, in shallow waters, fans must account for multidirectional water flow during the process of orientation. We found that fans did not orient perpendicular to either current or surge, but instead converged on an angle near perpendicular to the surge and did not converge on any angle relative to the current. In shallow waters, surge may exert more influence on fan orientation than current. When *Gorgonia* receives water flow from current, surge, and other directions at these velocities around the reef, a 75° angle to the surge may minimize hydrodynamic instability or maximize food acquisition.

Despite the convergence on 75°, *Gorgonia* still exhibited considerable variation in orientation. Substrate topography or the hydrodynamic complexity of water movement in shallow reefs could explain the range of orientations. The position, number, and size of coral colonies in a reef generate wakes, turbulence, recirculation forces, and other complications for modelling the flow of water (Hench & Rosman 2013).

Area of fan was not related to orientation. Larger fans were not oriented more

perpendicularly to surge direction than smaller fans. Temporal and directional variation of water flow may generate contradictory impulses in the process of passive reorientation by growth. Larger (and older) fans therefore would not orient closer to perpendicular despite having more time to grow. Future studies could quantify more detailed measurements of water movement around sea fans and investigate how they respond to daily, seasonal, or annual changes in the dominant directions of current and surge.

Organisms living in shallow reef waters must orient to maintain stability and obtain nutrients while withstanding complex, multi-directional water flow. Overall, our findings indicate that *Gorgonia* do orient relative to surge direction but not perpendicularly as predicted. The complexities of water movement in shallow reefs likely confuse their orientation process. While we examined only one coral genus, water movement may exert analogous influences on the development of other soft corals with other shapes and flexibility. Studying the interplay of hydrodynamics on coral spatial positioning is crucial for understanding their structuring role in reef communities.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

- Grigg, R. W. 1972. Orientation and growth form of sea fans. *Limnology and Oceanography* 17:185–92.
- Hench, J. L. and J.H. Rosman. 2013. Observations of spatial flow patterns at the coral colony scale on a shallow reef flat. *Journal of Geophysical Research: Oceans* 118:1142–56.
- Wainwright, S. A. and J. R. Dillon. 1969. On the orientation of sea fans (genus *Gorgonia*). *Biological Bulletin* 136: 130–9.

## EXAMINING LATERALIZATION IN TWO SPECIES OF CARIBBEAN REEF FISH

ALEXANDRA M. STENDAHL, ANGELA E. ORTLIEB, AND CORINNE R. VIETORISZ

Teaching Assistants: Michael B. Brown and Debora Goedert  
Faculty Editor: Celia Y. Chen

**Abstract:** Regional specialization in the brain, known as lateralization, is found in a wide range of species and often indicates enhanced cognitive ability. Lateralization is often expressed as a preference for one side of the brain for perceiving certain stimuli over another and can be assessed in fish by measuring turn direction bias. This study compares baseline lateralization in beaugregory damselfish (*Stegastes leucostictus*) and bluehead wrasse (*Thalassoma bifasciatum*), two species that belong to phylogenetically related families that coexist in Caribbean reefs but exhibit different life history traits. We assessed their baseline degrees of lateralization using a detour test. On average, beaugregory damselfish were 19% lateralized and bluehead wrasse were 24% lateralized according to their absolute lateralization indices. Neither species showed a preference for right or left, indicated by mean relative lateralization indices of 0 for both species. We found no difference in degrees of relative or absolute lateralization between species, despite their different social behaviors. The observed similarity in lateralization levels may be attributed to phylogenetic relatedness or similar environmental conditions. The results of our study can be used as a baseline to compare future work evaluating the effects of changing stimuli and environments on cognitive function of these Caribbean reef fish.

**Key words:** *Acanthochromis polyacanthus*, cognition, lateralization, *Pomacentrus chrysurus*

### INTRODUCTION

Brain asymmetry indicates regional task specialization in cerebral hemispheres and has been documented in a variety of different species, from humans to birds (Geswind 1985). A brain is asymmetrical if the two hemispheres are structurally unique and perform different functions (Bisazza et al. 1998). The primary benefit of lateralization is increased neural capacity, as specialization of one cerebral hemisphere for specific tasks leaves the other hemisphere free to perform other tasks. In fish, highly-lateralized individuals tend to escape from or avoid predation better, form more cohesive schools, and multitask more efficiently than fish with lower levels of lateralization. On an observable level, lateralization manifests as individuals demonstrating directional turn bias (more often turning right than turning left) as they turn to perceive stimuli with the specific brain hemisphere dedicated to assessing and responding to those stimuli (Bisazza and Brown 2011). By investigating turn bias in two common species of closely-related Caribbean reef fish, we evaluate baseline degrees of lateralization in these species and examine the potential relationships between lateralization and behavioral characteristics in these fish.

Baseline levels of lateralization have been shown to vary across species and in some cases, across individuals within the same population (Bisazza and Brown 2011, Bisazza et al. 1997). Studies suggest that highly-lateralized individuals may not perform better in all contexts; for example, stimuli coming from many directions at once might be better perceived by individuals with lower degrees of lateralization (Domenici et al. 2014). This suggests that, although high lateralization can enhance fitness in many contexts, the specific life histories of organisms may favor differing degrees of lateralization.

This study investigates the baseline levels of lateralization in the beaugregory damselfish (*Stegastes leucostictus*) and bluehead wrasse (*Thalassoma bifasciatum*). Many studies have documented lateralization in various species of damselfish, a reef-dwelling, herbivorous family of prey fish, best known for their extreme territoriality. Most male beaugregory damselfish guard their territories voraciously, fending off conspecifics, potential egg predators, and threats to their carefully-tended algal patches (Itzkowitz and Makie 1986). Our other focal species, the bluehead wrasse, is similarly herbivorous and vulnerable to predation, co-occurring with beaugregory damselfish in reefs throughout the

Caribbean. As juveniles, bluehead wrasse forage in small, loosely coordinated schools to avoid predation (Feddern 1965), a stark contrast to the solitary lives of beaugregory damselfish.

Beaugregories and bluehead wrasse belong to sister families and share similarities in habitat and territoriality, yet exhibit clear differences in foraging and social grouping strategies. This study investigates the baseline degrees of brain lateralization in these two species. If phylogeny or environment are primary determinants of lateralization, we might expect to see similarities in lateralization between beaugregory damselfish and bluehead wrasse; however, if their unique social and foraging behaviors require different degrees of lateralization, we might expect to find differences in baseline levels of lateralization between the species.

## METHODS

### *Catching fish*

We used handheld fishing nets to catch juvenile bluehead wrasse at Mary's Bay and juvenile beaugregory damselfish at South Hole Sound on Little Cayman Island. We brought the fish to the Central Caribbean Marine Institute (CCMI) laboratory for manipulations. We kept all bluehead wrasse together in a large tank but kept each beaugregory damselfish in its own small tank to avoid territorial displays and fights. We regularly cycled fresh ocean water through the tanks and aerated each tank with an oxygen bubbler. Once we had completed trials, we released all fish back into the ocean. No fish died throughout the course of our study.

### *Detour test*

To evaluate whether individual fish exhibit lateralization, we created a detour test, which is standard protocol for assessing lateralization (Bisazza et al. 1998). We constructed a simple maze inside a large tank filled with 4 cm of ocean water (Figure 1). For each trial, we placed the fish into the tank and let it freely explore and acclimate to the maze arena for 2 minutes. We then gently herded the fish back into the middle of the runway of the maze and waited for it to swim to the end of the tank on either side. We then recorded which direction it turned, as well as which side of the tank it swam to. We repeated this process 10 consecutive times for

each fish. To analyze each fish's directional decisions in the detour test, we calculated a relative lateralization index,  $L_R$  (Equation 1).

Equation 1:  $L_R = [(\text{Turn to the right} - \text{turn to the left}) / (\text{Turn to the right} + \text{Turn to the left})] * 100$   
Where "turns" to the right or left were quantified in 10 trials per individual fish, then averaged across fish.

This relative lateralization index allowed for comparisons of both the directionality and degree of lateralization within each species.  $L_R$  values fell between the extreme values of 100 (fish that turned right on all 10 trials) and -100 (fish that turned left on all 10 trials). To evaluate the degree of individual lateralization irrespective of left or right preference, we calculated the absolute value lateralization index,  $L_A$ , which corresponds to the absolute value of  $L_R$ .  $L_A$  allowed us to evaluate interspecific variation in degrees of lateralization. To compare the absolute lateralization indices between species, we ran a Wilcoxon signed-rank test on the  $L_A$  of each species.

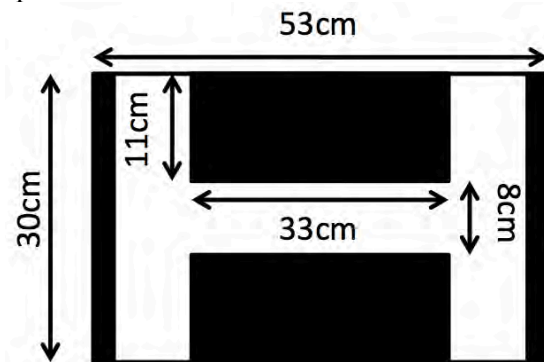


Figure 1. Detour test tank design. White area represents the open area the fish swims through and black area represents the area walled off to the fish.

## RESULTS

On average, beaugregory damselfish were 19% lateralized, with relative lateralization indices ranging between -40 and 60 (Figure 2; mean  $\pm$  SE =  $3 \pm 6$ ) and absolute lateralization indices ranging between 0 and 60 (Figure 3; mean  $\pm$  SE =  $19 \pm 5$ ). On average, bluehead wrasse were 24% lateralized, with relative lateralization indices ranging between -60 and 40 (Figure 2;

mean  $\pm$  SE =  $-12 \pm 10$ ) and absolute lateralization indices ranging between 0 and 60 (Figure 3; mean  $\pm$  SE =  $24 \pm 7$ ). Comparison between species showed no difference in absolute lateralization indices ( $Z = 0.56$ ,  $P = 0.57$ ).

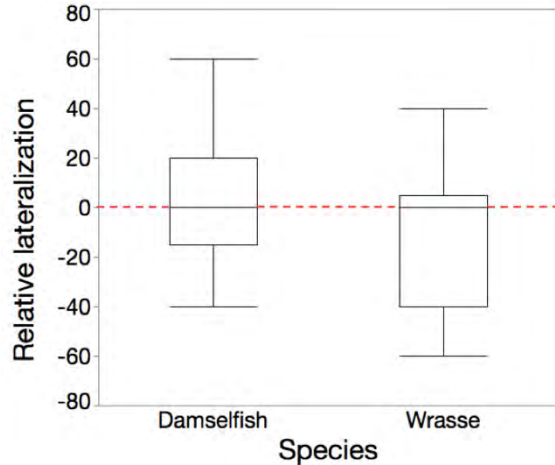


Figure 2. Relative lateralization ( $L_R$ ) shown by beaugregory damselfish and bluehead wrasse.  $L_R$  of 100 indicates that the fish turned left 100% of the time and  $L_R$  of -100 indicates that the fish turned right 100% of the time.

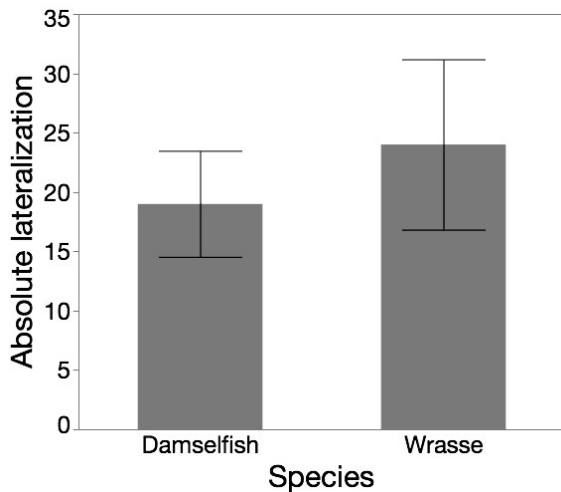


Figure 3. Absolute lateralization ( $L_A$ ) shown by beaugregory damselfish and bluehead wrasse.  $L_A$  of 0 indicates that the fish turned right and left an equal number of times and  $L_A$  of 100 indicates that the fish turned either left 100% of the time or right 100% of the time.

## DISCUSSION

Beaugregory damselfish exhibited similar lateralization levels to bluehead wrasse, despite having different foraging and social behaviors.

Beaugregory damselfish are primarily solitary and can be extremely territorial, while juvenile bluehead wrasse can form feeding aggregations and are territorial only as terminal males. One potential explanation for the observed similar levels of baseline lateralization between the species is that lateralization evolved early in phylogenetic history. Most vertebrates, and even some invertebrates, have brain asymmetry and exhibit behaviors consistent with lateralization (Rogers et al. 2002, Brown 2015). *Labridae* (wrasse) and *Pomacentridae* (damselfish) are considered sister families (Westneat et al. 2005). In this sense, if degree of lateralization is associated with phylogenetic relatedness, the two species should exhibit similar baseline degrees of lateralization. Secondly, if lateralization depends largely on environment, these species live together in the same reef, where presumably they experience similar abiotic conditions and predation threats. These comparable threats could be driving similar levels of baseline lateralization. Finally, lateralization has been shown to be highly context dependent; species tend to exhibit much higher degrees of lateralization when given predator or conspecific stimuli, so differences in the two species' degrees of lateralization may only be apparent if examined in response to species specific predator or conspecific stimuli (Domenici et al. 2014, Ferrari et al. 2015).

Further research should investigate how fish lateralization behavior changes with different stimuli and in varied environments. Similarly, fish lateralization has been shown to decrease with increased ocean acidification (Welch et al. 2014, Domenici et al. 2012). Future studies could build off of our work to establish whether beaugregory damselfish and bluehead wrasse exhibit increased behavioral lateralization with predation or conspecific stimuli, as well as in changing water quality. Furthermore, because most studies of fish lateralization focus on Indo-Pacific species that experience different environmental conditions and predation pressures, Caribbean fish species should be further evaluated.

Overall, the similar levels of lateralization exhibited by the beaugregory and bluehead wrasse indicate that phylogeny and environment may be more indicative of baseline lateralization



levels than their differing foraging and social behaviors. These findings provide insight into drivers of cognitive behavior across species and provide a springboard for further experiments on Caribbean reef fish lateralization.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

- Bisazza, A. and C. Brown. 2011. Fish Cognition and Behavior 298-324.
- Bisazza, A., L. J. Rogers, and G. Vallortigara. 1998. The origins of cerebral asymmetry: a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. *Neuroscience & Biobehavioral Reviews* 22(3): 411-426.
- Bisazza, A., R. Pignatti, and G. Vallortigara. 1997. Laterality in detour behaviour: interspecific variation in poeciliid fish. *Animal Behavior* 54:1273-1281.
- Brown, C. (2015). Fish intelligence, sentience and ethics. *Animal Cognition* 18(1): 1-17.
- Domenici, P., B. Allan, M. I. McCormick, and P. L. Munday. 2012. Elevated carbon dioxide affects behavioural lateralization in a coral reef fish. *Biol Lett* 8: 78-81.
- Domenici, P., B. J. Allan, S. A. Watson, M. I. McCormick, and P. L. Munday. 2014. Shifting from right to left: the combined effect of elevated CO<sub>2</sub> and temperature on behavioural lateralization in a coral reef fish. *PLoS One* 9(1).
- Feddern, H. A. 1965. The spawning, growth, and general behavior of the bluehead wrasse, *Thalassoma bifasciatum*. *Bulletin of Marine Science* 15(4): 896-941
- Ferrari, M. C., M. I. McCormick, B. J. Allan, R. B. Choi, R. A. Ramasamy, and D. P. Chivers. 2015. The effects of background risk on behavioural lateralization in a coral reef fish. *Functional Ecology* 29(12): 1553-1559.
- Geschwind, N., and A. M. Galaburda. 1985. Cerebral lateralization: Biological mechanisms, associations, and pathology: I. A hypothesis and a program for research. *Archives of Neurology* 42(5): 428-459.
- Itzkowitz, M. and D. Makie. 1986. Habitat structure and reproductive success in the beaugregory damselfish. *Journal of Experimental Marine Biology and Ecology* 97(3): 305-312.
- Rogers, L. J. 2002. Lateralization in vertebrates: its early evolution, general pattern, and development. In *Advances in the Study of Behavior* 31:107-161.
- Welch, M.J., S. A. Watson, J. Q. Welsh, M. I. McCormick, and P. L. Munday. 2014. Effects of elevated CO<sub>2</sub> on fish behaviour undiminished by transgenerational acclimation. *Nature Climate Change* 4: 1086-1089.
- Westneat, M. W. and M. E. Alfaro. 2005. Phylogenetic relationships and evolutionary history of the reef fish family Labridae. *Molecular Phylogenetics and Evolution* 36(2): 370-390.

## READY FOR THE RUNWAY: EVALUATING DECORATION IN *MICROPHRYS BICORNUTUS*

ALEC B. COBBAN, CLAYTON E. JACQUES, MARY M. PEDICINI, AND PAUL J. VICKERS

Teaching Assistants: Debora Goedert and Michael B. Brown

Faculty Editor: Celia Y. Chen

**Abstract:** Decorator crabs of the superfamily Majoidea are known for their diverse uses of decoration. Individual species of crabs decorate for many potential advantages. We investigated the decorating behavior of *Microphrys bicornutus* in laboratory manipulations by providing a variety of decoration materials and substrates. We also surveyed crabs in the field at night and day during low tides, measuring crab sizes, algae cover, and sand and algae substrates where the crabs were found. Crabs in the laboratory did not decorate their shells with provided materials or show any preference for substrates matching their coloration. In the field surveys, we found no evidence that *M. bicornutus* uses decoration for visual crypsis by color matching of their substrate. Although abundance and activity were higher at night, crab size and time of day did not affect percentage of algae cover on the carapace. Instead of visual crypsis by color matching, crabs may use disruptive camouflage to disguise body outline from predators.

**Keywords:** decorator crabs, disruptive camouflage, *Microphrys bicornutus*, visual crypsis

### INTRODUCTION

Decoration behavior - the attachment of material from the surrounding environment to an organism's body - serves many adaptive functions, such as avoiding predation or facilitating sexual selection (Ruxton and Stevens 2015). This behavior is commonly displayed in over 25 percent of all major metazoan phyla (Brooker et al. 2017), especially aquatic organisms. Among crabs of the superfamily Majoidea, many species demonstrate decoration behavior, but the adaptive function is often unclear (Ruxton and Stevens 2015). Members of the superfamily attach decorations to their exoskeleton via velcro-like setae (Brooker et al. 2017). Studies have found interspecific variation in the use for this decorating behavior, including noxious defense against predators (Stachowicz and Hay 1999) and chemical camouflage (Brooker et al. 2017). Additionally, decoration has been used for two distinct type of visual crypsis: matching camouflage where the crab coloration closely emulates the substrate color and disruptive camouflage where decorations disrupt the body outline of the crab (Wicksten 1992; Stevens and Merilaita 2009).

In our study, we investigated the use of decoration by the decorator crab *Microphrys bicornutus*. These crabs are active in day and night, living among light sand-colored rocks and dark-green turtle grass in shallow water. *M. bicornutus* decorates with dark algal clumps

over light sand-colored carapaces (Salazar 2013). Although *M. bicornutus* does occasionally consume its algal decorations, the potential usefulness of decoration for camouflage is unknown (Wicksten 1992).

We tested the hypothesis that decorator crabs attach decorations for visual crypsis. For choice tests in the laboratory, we predicted that crabs would select substrate backgrounds to match the color of their decorations and select decorations to match the color of their background. For our field surveys, we predicted that 1) when given the choice of decorating with different materials, crabs would select decorations most closely resembling the substrate; 2) when given the choice between different substrates, crabs would select the color most closely resembling the color of their decorations; 3) choice of substrate would be more important in the day, when visual information is more relevant to predators; 4) assuming that smaller crabs are more susceptible to predation, we expect smaller crabs to better match decorations to their substrate by minimizing color contrast. On the light-colored substrate the quantity of algal decoration would decrease with decreasing crab size, as smaller crabs are more vulnerable to predation and will decorate to minimize color contrast.

### METHODS

*Substrate and decoration choice experiments*

We collected 32 decorator crabs from the shore of Grape Tree Bay, Little Cayman. We identified the crustaceans as the speck-clawed decorator crab (*Microphrys bicornutus*, synonym *Microphrys bicornuta*). To test the crabs' ability to select a substrate matching their decorations, we presented 13 crabs with a substrate choice arena. We placed crabs into clear plastic tanks in which half of the substrate was brown (matched decorations) and half of the substrate was red (did not match decorations). After 6 hours, we recorded their position on the substrates. We also tested decoration preference to match underlying substrate. We scrubbed 14 crabs clean of algae and placed them into clear plastic tanks over a green substrate. We added 5 squares (~1 cm<sup>2</sup>) of green flagging tape and 5 squares of orange tape to each tank. To gauge crab interest for other decoration materials, two crabs received an additional 10 pieces of green paper (same material as the substrate), and one of the two received 5 pieces of green sponge. We examined the crabs for newly attached decorations after 12 hours.

#### *Crab field surveys*

We compared crab decorations by habitat substrate, time of day, and size with field surveys and measurements of crabs at low tide on the shore of Grape Tree Bay. We visited the shore during two periods of low tides, at night and during the day. During the surveys, we selected 5 random numbers between 0 and 40, corresponding to meter marks of a 40-meter transect. At each randomly selected meter mark, we placed two 1x1 meter quadrats, one quadrat in water over sand and algae-covered rocks (light substrate) 1 meter from the shoreline and another quadrat over turtle grass (dark substrate) between 2-5 meters from the shoreline. Because of the low tide, depths in the different quadrats was approximately equal. We surveyed each quadrat for active decorator crabs by visual and tactile inspection for 3-10 minutes. We counted the crabs collected per quadrat and photographed the crabs with an iPhone 7 camera at a consistent focal distance. We also measured the maximum width of each crab's carapace, the size measurement analyzed as a proxy for age by Brooker et al. (2017).

#### *Photograph analysis*

We counted the number of pixels on the carapace of each crab and the number of pixels occupied by algae (Fig 1) with ImageJ image analysis software (Rasband 2016). We only measured algal coverage of the carapace because of inconsistent leg positions within the photos and previous studies which found that related crabs decorate most on the carapace (Wortham 2012). We calculated the percentage of algal cover by dividing the number of pixels with algae by the total number of pixels.

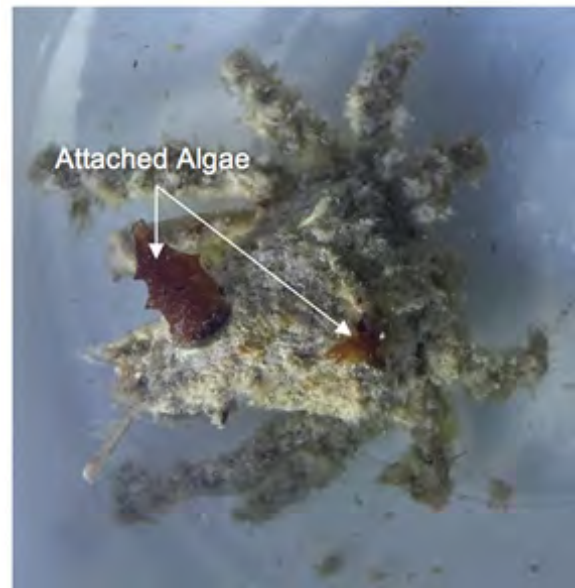


Figure 1: Photograph of *M. bicornutus* with algal decorations (arrows) attached to the carapace.

#### *Statistical analyses*

We arcsine-transformed the percentage of algal cover values and square root transformed the number of crabs (abundance) per quadrat to meet the assumption of normality. To examine the activity of crabs by time of day and substrate type, we compared the transformed abundance of crabs by the time of sampling (night/day) and the substrate type (light/dark) with a two-way ANOVA. To compare the decoration cover of the crabs by time of day and substrate, we conducted a two-way ANOVA of the transformed percentage of algal cover by the time of sampling (night/day) and substrate type (light/dark). To compare the decoration cover for crabs of different sizes, we performed a linear regression on the transformed percentage

of algal cover by the crabs' carapace width. All analyses were performed in JMP Pro 13.0.

## RESULTS

Decorator crabs in the laboratory displayed no association bias for red or brown substrate color; after 6 hours, we observed 7 crabs on the red and 6 crabs on the brown substrates. The crabs did not attach any of the artificial decorations after 12 hours, so no analysis was possible. However, while we scrubbed their carapaces, we did observe crabs reattach small algal clumps.

Crab abundance differed by time of day and substrate type. (Fig 2;  $F_{3,10} = 7.69$ ,  $P < 0.01$ ). Crabs were more abundant in the light substrate than in the dark (substrate:  $F_{1,10} = 6.31$ ,  $P = 0.03$ ), and more abundant at night than during the day ( $F_{1,10} = 7.94$ ,  $P = 0.02$ ). There was no interaction between these effects ( $F_{1,10} = 2.19$ ,  $P = 0.17$ ). Algal cover did not differ among crabs by time of day collected or by substrate (Fig 3;  $F_{3,91} = 1.39$ ,  $P = 0.25$ ). Algal cover did not vary by crab width in light or dark substrate (Fig 4; dark: slope =  $0.42 \pm 0.27$ ,  $P = 0.17$ ; light: slope =  $0.003 \pm 0.09$ ,  $P = 0.97$ ).

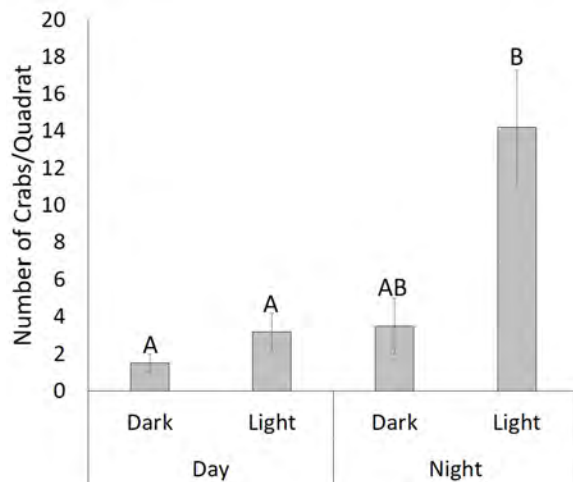


Figure 2: Mean abundance of crabs in a 1x1m quadrats on dark and light substrates and during day and night surveys. (mean  $\pm$  1 SE)

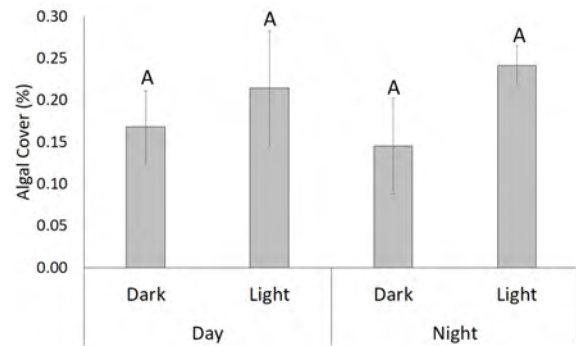


Figure 3: Mean percentage of algal cover on crabs by time of day and substrate type (mean  $\pm$  1 SE).

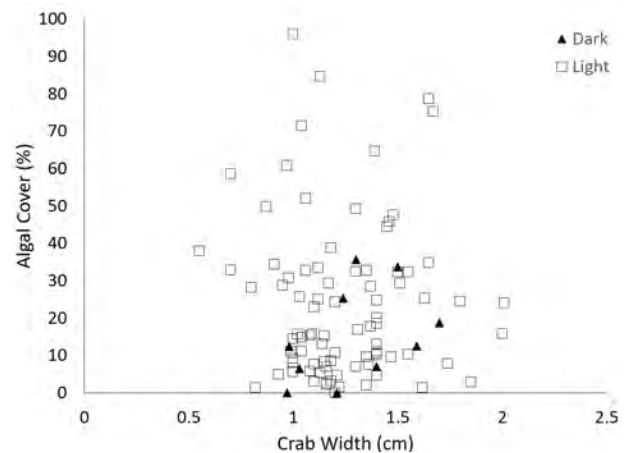


Figure 4: Percentage of algal cover based on crab width. White squares represent crabs found in quadrats over light substrate, and black triangles represent crabs found in quadrats over dark substrate.

## DISCUSSION

We observed no preference by the crabs to select substrates matching decoration color or to attach decorations to match substrate color. In fact, no crabs attached any of the materials placed in their tank regardless of its match with the substrate. This observation suggests that *M. bicornutus* do not perceive their own color contrast against substrates. However, the crabs may not have acclimated to laboratory conditions, and their responses might reflect abnormal behavior caused by the stress of an artificial environment. Moreover, we found more *M. bicornutus* active at night, consistent with the behavioral patterns of a nocturnal

organism. Crabs were also more abundant on the light rock substrate.

We found no difference in algal cover on crabs between the day or at night, and the match between crab and substrate was not improved during the day, when it might be more beneficial to hide from visually-oriented predators. Finally, we found no associations between crab size and algae cover regardless of substrate color. If decorations provide visual crypsis, the benefits of camouflage do not vary by the growth stage of the crabs.

Although we found evidence that *M. bicornutus* do not decorate for visual crypsis by color matching, if camouflage is a reason for the decoration behavior, disruptive camouflage is a more likely mechanism than crypsis. Because of lower light availability at night, nocturnal predators are likely not relying on visual cues to seek prey; thus, night-active prey like *M. bicornutus* would not decorate for visual crypsis. Nor did the crabs demonstrate any variation in diel behavior or substrate preference by their algal cover. Neither substrate was entirely light- or dark-colored, therefore crabs with patchy algal decoration may have resembled naturally occurring patterns in their surroundings, although not perfectly matched in coloration. The placement of dark algae clumps could break up the body outline of the crab and provide disruptive camouflage to the crab (Wortham 2012).

Further studies with laboratory manipulations should include better acclimation of the crabs to captivity to ensure normal behaviors and investigate preferences for natural decorations against natural substrates rather than artificial materials. Additionally, future studies should take into account the visual systems of *M. bicornutus* and its predators. Visual crypsis may function differently for organisms that perceive colors and patterns differently from humans, especially underwater.

#### ACKNOWLEDGEMENTS

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

- Brooker, R. M., E. C. Muñoz Ruiz, T L. Sih, *et al.* 2017. Shelter availability mediates decorating in the majoid crab, *Camposcia retusa*. *Behavioral Ecology* 29: 179–85.
- Rasband, W.S. 2016. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <https://imagej.nih.gov/ij/>.
- Ruxton, D. G. and Stevens, M. 2015. The evolutionary ecology of decorating behaviour. *Biol. Lett.* 11(6).
- Salazar, M. A. 2013. Functional aspects of behavior and morphology in the decorator crab *Microphrys bicornutus*. Florida Atlantic University, Boca Raton, FL.
- Stachowicz, J. J. and M. E. Hay. 1999. Reducing predation through chemically mediated camouflage: indirect effects of plant defenses on herbivores. *Ecology* 80: 495–509.
- Stevens, M. and Merilaita, S. 2009. Defining disruptive coloration and distinguishing its functions. *Philos Trans R Soc Lond B Biol Sci.* 364(1516):481–488.
- Wicksten, M. K. 1992. A Review and a Model of Decorating Behavior in Spider Crabs (Decapoda, Brachyura, Majidae). *Crustaceana* 64: 314–25.
- Wortham, J. L. 2012. The decorating behaviors of the spider crab *Libinia dubia*: size, sex, decoration selection, and disruptive camouflage. *Florida Scientist* 75: 259–78.

## REFUGE PREFERENCE IN BEAUGREGORY DAMSELFISH (*STEGASTES LEUCOSTICTUS*)

ALEXANDER W. COTNOIR AND BALTHASAR L. VON HOYNINGEN HUENE

Teaching Assistants: Michael B. Brown and Debora Goedert

Faculty Editor: Celia Chen

**Abstract:** Habitat selection is important for coral reef fauna, which are faced with a complex and diverse array of habitats. Refuge habitat selection has profound implications for both the survival, aiding in predator avoidance, and reproductive success of reef fishes. Beaugregory damselfish are known to prefer shelters with more openings, although relevant studies have only used artificial refuge structures to test refuge preference. To test whether preference for more openings holds for natural refuges, we studied beaugregory refuge selection among conch shells with varying numbers of holes. Laboratory preference tests produced mixed results, but field observations and manipulations showed clear preference among beaugregory for shells with more and larger holes. Larger shells were also preferred. Openings in refuge sites may provide important advantages, such as improving oxygen flow across eggs or allowing alternate predator escape routes. By comparing dimensions of shell holes with dimensions of occupant beaugregory, we found that the predator escape hypothesis is a plausible explanation for this preference. These results have profound implications for understanding microhabitat selection, and consequently territoriality and reproductive success of beaugregory damselfish.

**Key words:** Beaugregory damselfish, queen conch, refuge, shelter structure

### INTRODUCTION

Coral reefs provide diverse habitats and refuge sites for marine fauna. Some species select refuges in such a manner as to increase reproductive success and protection from predation. One such species is the beaugregory damselfish (*Stegastes leucostictus*). The beaugregory is typical of the genus *Stegastes*, with males defending small perennial territories (approx. 1- 2 m<sup>2</sup>) which provide food, shelter and sites for females to deposit eggs. Natural breeding/refuge sites within beaugregory territories are highly variable in structural complexity, which has been suggested to be an important aspect determining the attractiveness of a given territory and thus subsequent reproductive success and dominance (Cleveland et al. 2003, Santangelo et al. 2002, Itzkowitz 1991).

Previous studies investigating habitat preferences among beaugregory damselfish used artificial PVC- pipe structures to construct territory refuges. These studies found that males defending “open” sites (PVC assemblages offering multiple points of entry to a central cavity) exhibited twice the reproductive success of those males defending “closed” sites (PVC assemblages that were cave-like, with only one entrance). Such studies also found that male fish

preferred to defend open sites when given a choice between open and closed refuges (Itzkowitz and Makie 1986).

One limitation of these studies is that they relied on artificial PVC assemblages to simulate refuges found naturally on coral reefs. In our study, we used empty queen conch shells to determine whether the same patterns of preference hold for naturally-occurring structures. Preliminary observations revealed that beaugregory often use empty conch shells as refuges around coral reefs at Little Cayman Island. Further, these shells vary in number and area of openings; some contain holes from harvesting activity or natural degradation. We refer to a “hole” as any such opening in the shell aside from the valve. “Open” shells are those with relatively more and/or larger holes, while “closed” shells are more intact.

Following the findings of Itzkowitz (1991), we hypothesized that male beaugregory prefer open over closed shells as refuges and used a combination of field surveys and lab and field experiments to test this hypothesis. We predicted that occupied conch shells would contain more and larger holes than nearby unoccupied shells, and that beaugregory would occupy open shells rather than closed shells given a choice. If this preference were observed, it could stem from



several advantages of open shells, such as serving as a means to escape predators when cornered inside the shell (“alternative door hypothesis”). The alternative door hypothesis presents a simple, straightforward prediction; that holes in conchs used as beaugregory refuge sites must be large enough for the occupant fish to pass through. Therefore, we measured occupant fish size as well as hole size of shells in the field in order to determine if such a hypothesis is possible.

## METHODS

### *Site selection*

We conducted field surveys parallel to the back reef of Thornton’s Flats, South Hole Bay, and Grape Tree Bay surrounding the island of Little Cayman. Water depths ranged from 1-2 m in a habitat consisting of coral rubble. Beaugregory are the most common damselfish observed in such regions of the reef, with territorial males (distinguishable from females by their larger size and turf-defending behavior) guarding algal patches from a central shelter consisting of either coral rubble or vacant queen conch shells. For this investigation, we examined only beaugregory who established empty conch shells as their central refuge. Empty queen conch shells are plentiful around Little Cayman due to natural death and conch harvest. Conch shells also naturally exhibit a range in both the number and size of holes due to degradation.

### *Open vs. closed shell preference*

*Creation of open conch refuges.* For field and laboratory refuge preference experiments, we created open conch shell refuges, which contained two holes at the back of the conch shell: one large enough for an adult beaugregory to swim through and the other slightly smaller. We paired a closed conch shell (a fully intact shell of similar dimensions) with each open shell created. Open conch refuge sites thus allowed access to the shell interior from multiple entrances, while closed sites were cave-like with only one entrance.

*Laboratory shell preference test.* To test beaugregory refuge preference in a controlled environment, we collected 27 male beaugregory seeking refuge in conch shells in the wild from South Hole Sound. In the lab, we placed each

fish in an individual tank. Each tank was the same size and lighted equally, with opaque barriers to prevent visual contact between damselfish.

For all trials, we exposed damselfish to an open and closed conch shell using a standard dichotomous choice design (e.g., Williams and Mendelson 2011). For each of the first 11 fish, we conducted a choice trial, where the fish was placed in the center of a rectangular (30 cm x 80 cm) acrylic glass tank and allowed to freely access shells on both sides of the tank. The arrangement of the two shells on either side of the tank was randomly chosen, and both shells were placed in the tank 10 cm from either wall with the flange opening facing inward. We also placed the tank inside a large black plastic tub, to prevent the fish from reacting to our movements during experimental trials. We recorded over the course of 5 minutes: which shell the fish first investigated, how many times the fish entered into or sought refuge beneath the open and closed shell, and the duration of time in which the fish spent seeking refuge at each shell (designated as swimming directly underneath, alongside, or inside the shell). We calculated the strength of preference (SOP) for open shells using the following formula from Williams and Mendelson (2011):  $SOP = (T_O - T_C) / (T_O + T_C)$ , where  $T_O$  is the time spent seeking refuge at the open shell, and  $T_C$  is the time spent seeking refuge at the closed shell.

For the remaining 16 individuals, we conducted a second experiment to account for possible side bias, which could confound results of the choice for open and closed shells. We performed dichotomous choice tests as previously described, but at the end of the 5 minutes we placed the individuals back in the center of the tank, swapped sides of the shells, and repeated the trial for another 5 minutes.

After the final trial for each fish, we recorded which section of the tank the fish was positioned within. Fish position could fall into three categories: center of the tank (not seeking refuge at either shell) or seeking refuge at the open or closed shell (given the metrics used above to define taking refuge). We then presented the fish with a perceived threat by hitting the center of the top of the tank with an extended arm, recording which area (center,

open shell, or closed shell) of the tank the fish moved to.

*Field surveys.* To determine if beaugregory preferentially seek refuge in more open shells in a natural setting we swam a total of four 70 m transects: one in both Thornton's Flats and South Hole Sound, and two at Grape Tree Bay parallel to the fringing reef. At all three sites, we recorded all queen conch shells occupied by beaugregory within 4 m to the right and left of the transect, measuring characteristics of shells used as refuge and unoccupied conch shells. We only measured unoccupied conch shells if the largest opening to the interior of the shell was: 1) not clogged with debris, 2) facing in a direction accessible by beaugregory, and 3) large enough for a beaugregory to enter the shell. Shells were considered refuges if beaugregory were seen moving in and out of, guarding, or retreating into them when we swam by. To assess the degree of "openness" of the occupied and unoccupied shells, we recorded the number of holes and measured the length and width of the largest hole using a caliper. We also recorded the age class (juvenile or adult) of the fish occupying the shell, as well as shell length to determine if shell size influences habitability.

*Field shell preference test.* To further assess if beaugregory prefer open conch shells as refuge in a natural setting over fully intact/closed conch shells, we placed fifteen pairs of similarly-sized conch shell refuges side by side in the center of fifteen beaugregory territories where fish did not have conch shell refuges in Grape Tree Bay. Given that beaugregory territories measure 1-2 meters across, shell pairs were placed at least 5 meters apart from one another to ensure that all pairs could be occupied simultaneously (Itzkowitz 1991). For each pair of conch shells placed within a beaugregory territory, one was an open shell with two large holes chiseled into the back of the flange, and the other was an intact conch shell. We placed all pairs of shells with their flange openings facing towards shore. We then returned 1 day later to record which of the shells was being utilized as a refuge by beaugregory.

#### *Hole size*

*Fish measurement.* To determine whether fish choose conch shell refuges with holes large

enough to provide extra escape routes, we also caught 14 beaugregory occupying conch shells along the fringing reef at South Hole Sound and measured their body dimensions. For each fish, we measured the vertical height (distance between the dorsal and pelvic fins) and body width at the widest portion of the body (Figure 1). We also recorded the length and width of the largest hole in the shell in which they took refuge, in order to compare body dimensions to the size of potential escape routes.

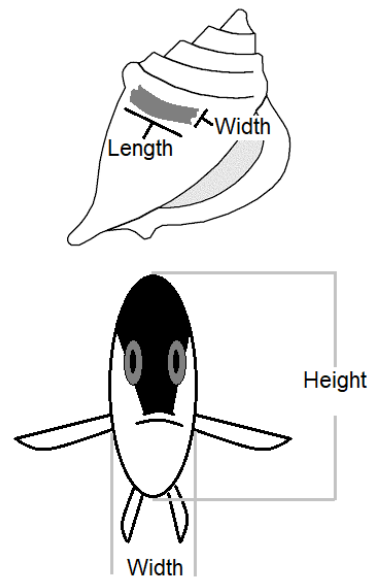


Figure 1. Diagram of measurements recorded for shell holes and corresponding fish.

#### *Data analysis*

For field observation data, we used a Wilcoxon test to compare each of several variables between occupied and unoccupied shells: number of holes, area of largest hole, and shell length. To analyze the laboratory shell preference test data, we used the above SOP index to assess refuge preference. For each of the two experiments, we compared the mean index score against zero using a Wilcoxon Signed-Rank test. We used Wilcoxon nonparametric tests to investigate the possibility of confounding variables affecting shell preference, including side of tank and fish life stage. To contrast the dimensions of the largest shell holes with occupant fish body dimensions, we visually inspected the relationship between fish and hole length, and the fish and hole width using scatterplots.

## RESULTS

*Open vs. closed shell preference*

*Laboratory shell preference test.* Strength of preference did not vary depending on the side of the tank containing the open shell for either the first or second preference experiments in the lab (experiment 1: Wilcoxon,  $S = 17$ ,  $Z = 1.22$ ,  $P = 0.22$ ; experiment 2: Wilcoxon,  $S = 230$ ,  $Z = 0.86$ ,  $P = 0.39$ ). In experiment 1, life stage had a marginally significant effect on preference (Wilcoxon,  $S = 41$ ,  $Z = 1.92$ ,  $P = 0.06$ ). In experiment 2, life stage had no effect on preference (Wilcoxon,  $S = 209.5$ ,  $Z = 0.0$ ,  $P = 1.0$ ). Experiment 1 involved 5 adults and 6 juveniles, while experiment 2 involved 7 adults and 9 juveniles.

In experiment 1, beaugregory preferred open shells (Wilcoxon Signed-Rank, test statistic = 26,  $P = 0.019$ ). In experiment 2, shell type had no effect on preference (Wilcoxon Signed-Rank, test statistic = 25.5,  $P = 0.59$ ). Across trials in experiment 2, fish preference for open shells was higher before the shell positions were switched, although not significantly so (Wilcoxon,  $S = 249$ ,  $Z = 1.69$ ,  $P = 0.091$ ).

Across both experiments, we observed nine instances of beaugregory entering the flange of the open shell (accounted for by 6 different fish). No fish entered the flange of the closed shell.

When responding to a threat, fish tended to seek refuge in the closest shell. Out of 9 times in which fish ended a trial near a shell, only once did the fish seek refuge at the shell on the opposite side of the tank. In the 5 times when fish ended trials at the center of the tank, all 5 fish responded to the threat by seeking refuge at the open shell.

*Field surveys.* We found 60 shells, of which 32 were occupied by a beaugregory. Occupied shells had more holes than did unoccupied shells (Wilcoxon,  $S = 467$ ,  $Z = 6.03$ ,  $P < 0.001$ ; Figure 2), and had a larger area of the largest hole even when shells without holes were excluded (Wilcoxon,  $S = 304.5$ ,  $Z = 3.12$ ,  $P = 0.002$ ; Figure 3). Occupied shells were also significantly longer than unoccupied shells (Wilcoxon,  $S = 596$ ,  $Z = 3.85$ ,  $P < 0.001$ ).

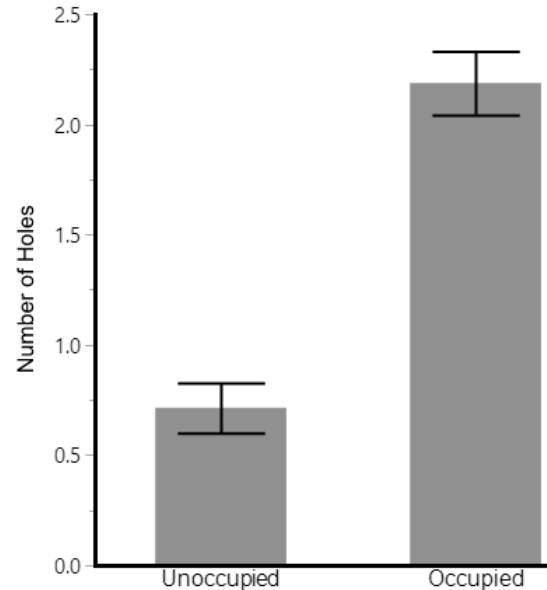


Figure 2. Number of holes in shell (excluding valve) by occupancy. Unoccupied mean  $\pm$  SE =  $0.71 \pm 0.11$ ; Occupied mean  $\pm$  SE =  $2.18 \pm 0.15$ .

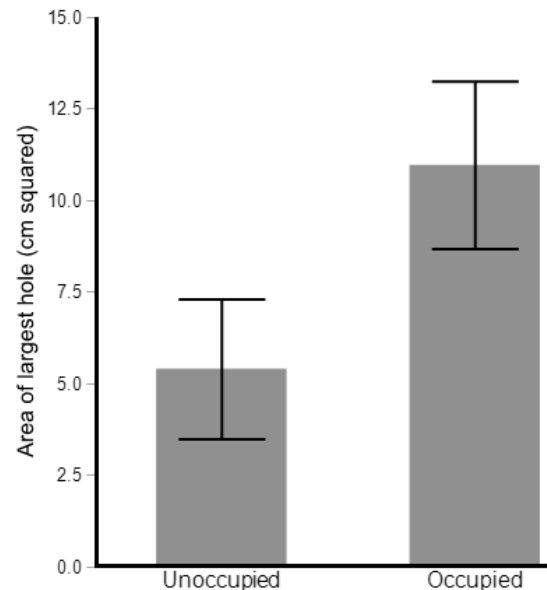


Figure 3. Area of largest hole (excluding valve) by occupancy. All shells with no holes were excluded from calculations. Unoccupied mean  $\pm$  SE =  $5.40 \pm 1.91$ ; Occupied mean  $\pm$  SE =  $10.97 \pm 2.29$ .

*Field shell preference test.* Of 15 pairs of shells, 10 pairs were unoccupied by beaugregory 1 day later (Table 1). In 4 pairs, only the open shells were occupied, and in 1 pair both shells were occupied. All occupants were juveniles. One closed shell had been flipped, presumably due to wave action.

Table 1. Occupation status of shell pairs placed in the field after 1 day.

Shells occupied after 1 day	# pairs
Neither	10
Open only	4
Closed only	0
Both	1

#### Hole size

Of 12 occupied shells with holes, the occupant fish was small enough to fit through the largest hole (Figure 4). On several occasions, fish evaded capture by escaping through a hole.

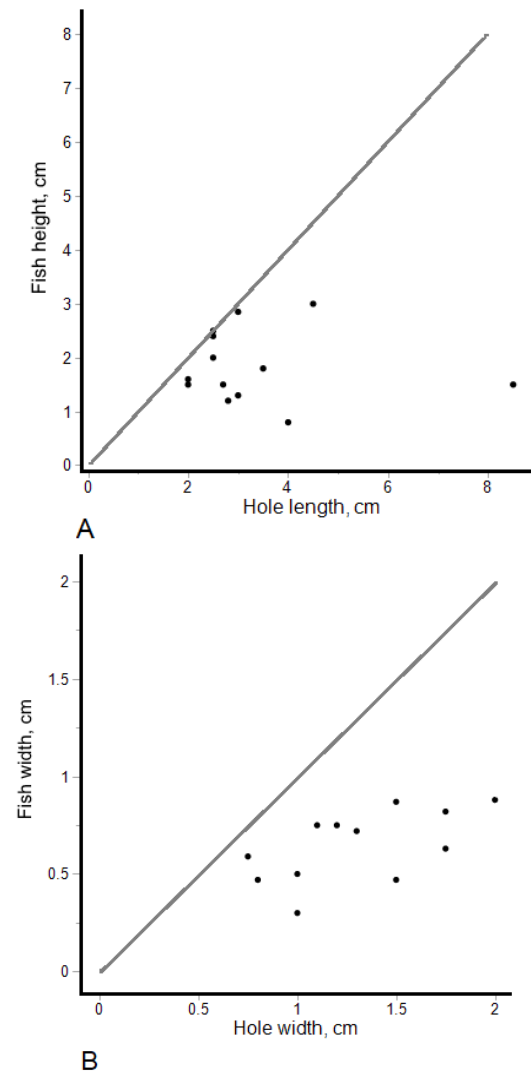


Figure 4. Fish and shell hole size comparisons: A) fish and hole height; B) fish and hole width. Line represents equal fish and hole sizes.

#### DISCUSSION

Our study shows that male beaugregory preferentially seek refuge in open over closed conch shells, consistent with our hypothesis. Additionally, beaugregory chose shells with both more and larger holes. A possible explanation for this preference is the “backdoor hypothesis”: holes in the shell provide alternate escape routes. Our results support this hypothesis, since shell holes tend to be larger than the fish occupying the shell (Figure 4). Several fish used these holes to evade our capture attempts. Additionally, shell holes may allow water to flow through the shell, which may prevent shells from being moved by surge or may improve the flow of oxygen over eggs laid within. These explanations are not mutually exclusive. To test such hypotheses, future studies could conduct shell preference trials in which males are presented with two open shells: one with a hole large enough for the individual to swim through, and the other with multiple smaller holes too small for the individual to pass through but of equivalent total size.

Results from our first round of lab shell preference tests also indicate that beaugregory prefer to seek refuge in open over closed shells, although no preference emerged in the second experiment. Beaugregory preferred the open shell in the first five minutes of all trials, but not when shell positions were switched during the second round of tests. We recognize that several confounding factors may have impacted shell preference in a lab environment. Certain behaviors indicated that some individuals were investigating their reflections on the tank wall. During the second laboratory experiment, it is also possible that once individuals located their preferred shell refuge in the first trial and associated with that side of the tank, they returned to this site after the shells were switched. This degree of site fidelity is plausible, given that males defend permanent territories (Itzkowitz and Makie 1986).

We also observed other behavioral indications of preference for the open shell (Table 2). Beaugregory never entered the closed shell cavity during preference tests, while entering the cavity of the open shell nine times. When presented with a simulated threat, beaugregory always sought refuge at the open

shell if they were positioned in the center of the tank. This finding indicates that open refuges may be preferred in cases when potential predators are present, supporting the backdoor hypothesis.

Interestingly, only juvenile male beaugregory occupied the empty conch shells placed in the field. This finding may indicate that younger, less territorial males are effectively “in the housing market,” whereas older, sexually mature, and reproductively active males are more steadfast in their site fidelity. This result is consistent with findings of previous studies where adult males took longer periods of time to occupy artificially created PVC refuges and only occupied PVC refuges if the site was better in quality than their current refuge site (Itzkowitz 1991).

Our findings provide substantial evidence that the number of entrances to a refuge is an important characteristic determining the attractiveness of sites where beaugregory seek shelter and establish territory. Previous studies have found strong correlations between reproductive success of beaugregory males and differences in the structure of artificial refuges (Itzkowitz and Makie 1986). Given the strong preference we observed for open natural refuge sites, our findings may provide further insight into the factors determining the reproductive success of the species. Other research suggests that structural complexity within beaugregory territories greatly impacts social interactions, including a male’s attractiveness to potential mates, and dominance hierarchies among neighboring individuals (Cleveland et al. 2003). Although these studies examine beaugregory territories in their entirety, given the strong evidence we found for selectivity in the structure of refuge sites, our study also lays groundwork for future investigations of social interactions in the species.

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#### AUTHOR CONTRIBUTIONS

Both authors contributed equally to the creation of the final project.

#### LITERATURE CITED

- Cleveland, A. L., Ludlow, A. M., Itzkowitz, M. 2003. Dominance relationships between male territorial neighbors in the beaugregory damselfish (*Stegastes leucostictus*). *Journal of Animal Behaviour* 140: 1021-1037.
- Haley, M. P. & Christian R Müller, R. C. 2002. Territorial behaviour of beaugregory damselfish (*Stegastes leucostictus*) in response to egg predators. *Journal of Experimental Marine Biology and Ecology* 273: 151-159.
- Itzkowitz, M. 1991. Habitat selection and subsequent reproductive success in the beaugregory damselfish. *Environmental Biology of Fishes* 30: 287-293.
- Itzkowitz, M., Ludlow, A., and Haley, M. 2000. Territorial boundaries of the male beaugregory damselfish. *Journal of Fish Biology* 56: 1138-1144.
- Itzkowitz, M. & Makie, D. 1986. Habitat structure and reproductive success in the beaugregory damselfish. *Journal of Experimental Marine Biology and Ecology* 97: 305-312.
- Santangelo, N., Itzkowitz, M., and Richter, M. 2002. Resource attractiveness of the male beaugregory damselfish and his decision to court or defend. *Behavioral Ecology* 13: 676- 681.
- Conch image in Figure 3 sourced from <http://clipart-library.com/clipart/5TRX8x6Lc.htm>.
- Williams, T. H. & Mendelson, T. C. 2011. Female preference for male coloration may explain behavioural isolation in sympatric darters. *Animal behavior* 82: 683-689.

## CORAL ANTI-PREDATOR RESPONSE VARIES BASED ON STIMULUS REPETITION AND SPECIES

GRACE S. CALLAHAN, HANNAH I. HOFFMAN, AND MAANAV JALAN

Teaching Assistants: Debora Goedert and Michael Butler Brown  
Faculty Editor: Celia Chen

**Abstract:** Coral colonies face predation pressure from a variety of corallivores and can evade this threat by retracting their polyps. This retraction is mediated by a nerve net, which is a simple type of nervous system shared by many phyla. We evaluated polyp retraction for three coral types on Little Cayman Island, analyzing recovery times after tactile stimulation to determine whether this response remains constant or whether it can vary based on stimulus repetition or coral type. We found that polyps of all coral types retracted for longer durations upon the second stimulation than the first, indicating that corals sensitize to repeated tactile stimuli. *Porites porites* exhibited significantly longer first and second recovery times than *Porites astreoides*; however, we found no difference in recovery times between purple and white *P. porites*. First and second recovery times were positively correlated for all three coral types. Lastly, *P. astreoides* displayed relatively stable recovery times for four stimulation events after sensitizing to the first. These findings suggest that corals exhibit varying reactions to predator cues despite sharing an ostensibly simple mechanism for responding to their environments.

**Key words:** coral, nerve net, polyp retraction, *Porites astreoides*, *Porites porites*, predation

### INTRODUCTION

Organisms employ many strategies to evade predators. The magnitude of these responses often varies based on the recurrence of threatening stimuli and on characteristics such as phylogeny and morphotype. It is little understood whether species lacking sophisticated cephalization exhibit such patterns, as they display simple evasion techniques and do not have means to centrally process stimuli. Cnidarians, a group including jellyfish, corals, and sea anemones, represent one such phylum. These organisms have loose networks of interconnected neurons called nerve nets, which lack centralized processing capabilities but allow signals from sensory stimuli to be transmitted across an individual (Arendt et al. 2016).

Coral colonies face predation pressure from a variety of corallivorous marine organisms including fishes, annelids, crustaceans, echinoderms, and mollusks (Rotjan and Lewis 2008). In response to these threats, coral colonies employ polyp retraction as a defense strategy, which is mediated by their nerve nets (Horridge 1957). Upon receiving a predator cue, such as the touch of a browsing corallivore, a polyp sends a signal across the colony, leading to a coordinated retraction of neighboring polyps (Kawaguti 1964; Anderson 1976;

Grimmelikhuijzen & Westfall 1995). After a period of time, the retracted polyps extend. Repeated tactile stimulation may be a particularly threatening cue for a coral colony, indicating the presence of a corallivore attempting to feed on its polyps. Corals may be able to respond differently to incidental contacts from non-threatening organisms and targeted, repetitious touches from a corallivore.

Coral species may employ different polyp retraction strategies given their unique morphologies and ecologies. For instance, a heavily predated species might exhibit sustained periods of retraction to minimize polyp loss. Alternatively, coral colonies that are frequently predated may reduce retraction period to limit the metabolic losses incurred by concealing photosynthetic zooxanthellae (Swain et al. 2015). Some species might rely on the photosynthesis more than others, resulting in polyp retraction differences.

Within coral species, variations in physiological stresses may also lead to changes in responses to predation cues. One such physiological stress is bleaching, different levels of which may be associated with two *Porites porites* morphotypes: the white and the purple (Correia K., pers. comm.). When the white morphotype expels zooxanthellae after a



bleaching event, bacteria normally housed deep within its polyps may rise to the surface of the epidermal layer, resulting in a purple color (Correia K., *pers. comm.*). Zooxanthellae can provide upwards of 90% of a colony's nutrition, so coral colonies without these algae are unlikely to meet their energetic needs (Berkelmans and van Oppen 2006). Therefore, purple *P. porites* may extend their polyps more quickly after retraction to maximize heterotrophic gains and meet their energetic needs even without zooxanthellae.

In this study, we investigated patterns in coral response to simulated predation cues. We presented extended polyps of coral colonies with a repeated tactile stimulus and measured their recovery times, anticipating one of two possible results. Recovery times may not vary based on stimulus repetition, indicating that corals are not conditioned by previous experience. Alternatively, corals may show conditioned responses, either habituating to repeated but harmless stimuli by recovering faster from repeated stimulation or sensitizing to potentially harmful stimuli by increasing recovery time on repeated stimulation. Two coral species—*P. porites* and *P. astreoides*—may not exhibit the same polyp retraction behaviors due to differences in predation pressures, resulting from variations in morphology or palatability. These patterns may also differ between species given genetic divergence. Patterns of recovery times may differ between *P. porites* morphotypes due to altered physiological needs.

## METHODS

We investigated patterns in polyp recovery using three coral types in Grape Tree Bay, Little Cayman Island between March 1 and 3, 2018: the white *P. porites* morphotype, the purple *P. porites* morphotype, and the green *Porites astreoides* morphotype. We chose these types because they extend their polyps during the day. When we encountered one of these coral colonies, we probed it with a cylindrical rod (0.6 cm diameter) to mimic a threatening stimulus. Selected corals inhabited the fringing reef and did not have any retracted polyps before stimulation. The same researcher performed stimulations such that they were consistent across all trials. We recorded the time of this

first stimulus and the time at which all retracted polyps subsequently extended, defining the difference in these times as the first recovery time. Immediately after recovery, we probed the coral again in the same location to stimulate the same polyps. We again recorded the time of the second stimulus and the time at which all retracted polyps fully extended, defining the difference in these times as the second recovery time.

We ran two linear mixed-effect models: one to determine the effect of stimulus repetition and species on the polyp recovery time and the other to determine the effect of stimulus repetition and morphotype on *P. porites* polyp recovery time. The former model used recovery times as the predicted value; stimulus number, *P. porites* morphotype, and the interaction between stimulus number and *P. porites* morphotype as fixed effects; and coral identity as a random effect. The latter model used recovery times as the predicted value; stimulus number, species, and the interaction between stimulus number and species as fixed effects; and coral identity as a random effect. For both models, we tested all pairwise comparisons among means using Tukey's HSD test. To assess how much individual variation explained recovery time variation in each model, we divided the covariance parameter estimate for coral identity by the sum of this value and the covariance parameter estimate for the residual.

For each of the three coral types, we ran a linear regression on first and second recovery times to evaluate whether individuals that exhibited longer first recovery times also exhibited longer second recovery times. For all analyses, we log-transformed recovery times to meet the assumption of normality. We ran analyses in JMP Pro 13 (SAS Institute Inc 2007).

To evaluate the effect of multiple, consecutive stimulations on recovery times, we probed five *P. astreoides* five times each and recorded recovery times, following the same stimulation protocol outlined previously. Due to time restraints, we could not complete this test for all three coral types nor could we test more than five *P. astreoides*. Therefore, we plotted curves for each individual showing recovery

times over the five stimulation events and identified trends qualitatively.

## RESULTS

Each of the 36 coral colonies that we tested (11 white *P. porites*, 10 purple *P. porites*, and 15 *P. astreoides*) exhibited longer second recovery times than first. For both *P. porites* morphotypes and for both coral species, second recovery times were longer than first recovery times (Figure 1; Tukey's HSD,  $P < 0.05$ ). *P. porites* displayed significantly longer first and second recovery times than those of *P. astreoides* (Figure 1a; Tukey's HSD,  $P < 0.05$ ). White *P. porites* and purple *P. porites* exhibited similar first recovery times and second recovery times (Figure 1b; Tukey's HSD,  $P > 0.05$ ). Individual variation accounted for 64% of recovery time variation between species and 67% of recovery time variation between morphotypes.

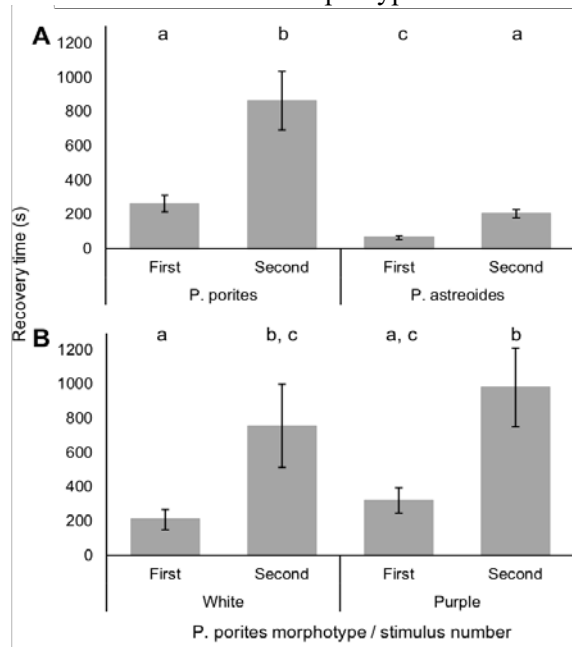


Figure 1. First and second polyp recovery times of two coral species (A) and two *P. porites* morphotypes (B). Within comparisons of species and morphotypes, different letters denote treatments that were significantly different (Tukey's HSD;  $P < 0.05$ ). (N = 11 white *P. porites*, 10 purple *P. porites*, and 15 *P. astreoides*)

Second response times were positively associated with first response times for all three coral types (Figure 2). These relationships were significant for purple *P. porites* and *P. astreoides* and tended towards significance for

white *P. porites* (Figure 2; white *P. porites*: slope  $\pm$  SE =  $0.35 \pm 0.18$ ,  $P = 0.09$ ,  $r^2 = 0.29$ ; purple *P. porites*: slope  $\pm$  SE =  $0.95 \pm 0.18$ ,  $P < 0.001$ ,  $r^2 = 0.78$ ; *P. astreoides*: slope  $\pm$  SE =  $0.47 \pm 0.20$ ,  $P < 0.05$ ,  $r^2 = 0.30$ ).

The five *P. astreoides* increased recovery times upon second stimulation and had consistent recovery times for the subsequent three stimulation events (Figure 3). However, the difference between recovery times for first and subsequent stimuli was not constant across individuals (Figure 3).

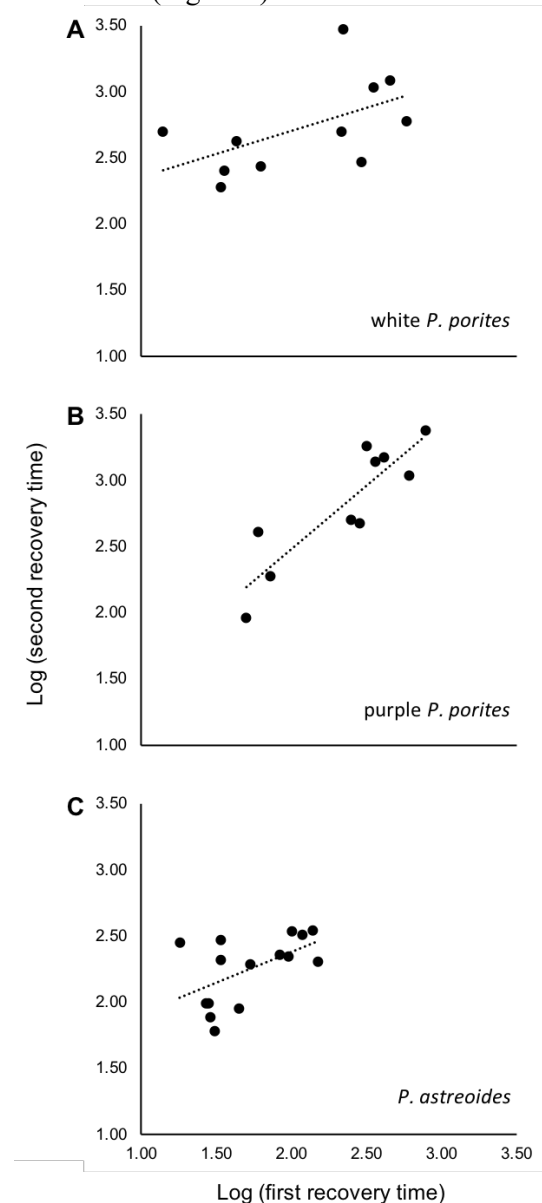


Figure 2. Relationships between first and second polyp recovery times of three coral types when exposed to a tactile stimulus.

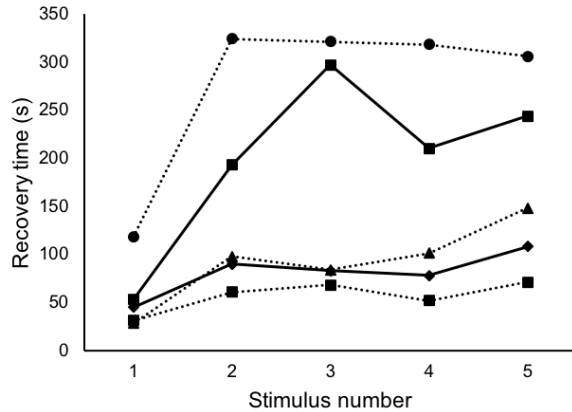


Figure 3. Polyp recovery times of five *P. astreoides* corals when exposed to a repeated tactile stimulus. Each line represents an individual coral.

## DISCUSSION

Across all three coral types, we found that second recovery times were significantly longer than first recovery times. This finding indicates that corals can be conditioned to stimuli despite having only nerve nets — the most primitive nervous system — to process them. This trend may be so pervasive across corals because repeated stimulation confirms the presence of a legitimate threat. Further studies should be conducted to provide a proximate explanation for coral sensitization to tactile stimuli, such as long-term potentiation in nerve net synapses.

We found that both the first and second recovery times varied significantly between *P. porites* and *P. astreoides*; this result implies that the two species have somewhat divergent nervous systems, which may have evolved for many reasons. Since *P. porites* experiences higher predation pressure than *P. astreoides*, longer recovery periods may limit polyp losses to predators (Littler et al. 1989). *P. astreoides* may rely more heavily on zooxanthellae for nutrition than *P. porites* such that *P. astreoides* will incur greater costs for longer recovery times, as the photosynthetic capabilities of zooxanthellae diminish when polyps retract (Levy et al. 2006).

The finding that first and second recovery times did not differ between the *P. porites* morphotypes indicates that the bleaching response associated with the purple morphotype may not influence how polyps react to threatening stimuli. The similarity in recovery times of the morphotypes also suggests that

purple *P. porites* does not increase heterotrophic intake to compensate for its lack of zooxanthellae, as previous research has shown for other species in the *Porites* genus (Grottoli et al. 2006). It is also possible that the bacteria responsible for the purple coloration photosynthesize and thus assume the role of zooxanthellae in the coral. Further studies should be conducted to understand the effects of purple coloration on the function of *P. porites*.

Individual colony variation accounted for much of the recovery time variation observed; this result provides further evidence that polyp retraction is not a fixed behavior but rather can vary between coral colonies. Furthermore, we observed positive associations between first and second recovery times across all coral types, suggesting that individuals within a type varied in sensitivity to the stimulus. Varying sensitivity levels and thus the high degree of individual variation observed could result from small genetic differences that lead to disparities in nerve excitability. Alternatively, environmental factors such as availability of sunlight and nutrients may cause sensitivity discrepancies, as these environmental factors are known to influence the timing of coral polyp retraction (Sebens and DeRiemer, 1977). To determine the degree to which variation within individual coral colonies causes differences in reactivity, future studies could investigate coral sensitivity in a controlled environment.

All five *P. astreoides* colonies demonstrated similar recovery time patterns for five stimulation events, with an initial increase in recovery time followed by little disparity in subsequent recovery times. However, there was some variation in the magnitude of differences between first and subsequent recovery times. The consistency of the curves suggests a possible upper limit on retraction time for this species, beyond which polyps may not benefit from retraction or are physiologically incapable of longer retraction. Perhaps this duration— on an individual basis— balances the benefit of predator evasion with the cost of no longer feeding or allowing zooxanthellae to photosynthesize optimally. The variation in magnitude between first and subsequent recovery times between these individuals provides additional evidence for sensitivity

differences even within species. Future studies could compare similar curves for multiple coral species to determine if they follow comparable trends.

This study system presents many opportunities to further elucidate behaviors of animals with simple nervous systems. Number of polyps retracted and time over which polyp retraction and extension occur can be used as alternative metrics for retraction behavior. Such variables could be compared across coral colonies of various sizes and in various light and food conditions to better understand the tradeoffs of retraction. Additionally, stimulations could be presented at different parts of coral colonies to study communication across a nerve net rather than just a small area. Individuals with simple nervous systems can exhibit extensive behavioral variation, and investigation of these patterns can elucidate how they interact with their environments.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

#### LITERATURE CITED

- Anderson, P.A. 1976. An electrophysiological study of mechanisms controlling polyp retraction in colonies of the scleractinian coral *Goniopora lobata*. *Journal of Experimental Biology* 65: 381-393.
- Arendt, D., M. A. Tosches, and H. Marlow. 2016. From nerve net to nerve ring, nerve cord and brain — evolution of the nervous system. *Nature Reviews Neuroscience* 17: 61-72.
- Berkelmans, R. and J. H. van Oppen. 2006. The role of zooxanthellae in the thermal tolerance of corals: a ‘nugget of hope’ for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences* 273: 2305–2312.
- Grimmelikhuijzen, C.J.P. and J. A. Westfall. 1995. The nervous systems of Cnidarians. *The Nervous Systems of Invertebrates: An Evolutionary and Comparative Approach*. Pages 7-24. Birkhauser Verlag, Basel.
- Grottoli, A. G., L. J. Rodrigues, and J. E. Palardy. 2006. Heterotrophic plasticity and resilience in bleached corals. *Nature* 440:1186-1189.
- Horridge, G.A. 1957. The co-ordination of the protective retraction of coral polyps. *Philosophical Transactions of the Royal Society B* 240: 495-529.
- JMP, Pro 13. SAS Institute Inc., Cary, NC, 1989-2007.
- Kawaguti, S., 1964. Electron microscopic study of the nerve plexus in the polyp of a reef coral. *Proceedings of the Japan Academy* 40: 121-124.
- Levy, O., Z. Dubinsky, Y. Achituv, and J. Erez. 2006. Diurnal polyp expansion behavior in stony corals may enhance carbon availability for symbionts photosynthesis. *Journal of Experimental Marine Biology and Ecology* 333:1-11.
- Littler, M. M., P. R. Taylor, and D. S. Littler. 1989. Complex interactions in the control of coral zonation on a Caribbean reef flat. *Oecologia* 80:331-340.
- Rotjan, R. D., S. M. Lewis. 2008. Impact of coral predators on tropical reefs. *Marine Ecology Progress Series* 367: 73-91.
- Sebens, K.P. and DeRiemer, K. 1977. Diel cycles of expansion and contraction in coral reef anthozoans. *Marine Biology*. 43: 247-256.
- Swain, T. D., J. L. Schellinger, A. M. Strimaitis, and K. E. Reuter. 2015. Evolution of anthozoan polyp retraction mechanisms: convergent functional morphology and evolutionary allometry of the marginal musculature in order Zoanthidea (Cnidaria: Anthozoa: Hexacorallia). *BMC Evolutionary Biology* 15:1-19.

## META-ANALYSIS OF COOPERATIVE “SCIENCE” AMONG UNRELATED *HOMO SAPIENS*

CLAYTON E. JACQUES

Teaching Assistants: Debora Goedert and Michael Butler Brown

Faculty Editor: Celia Y. Chen

*Abstract:* Despite the benefits of cooperation, cooperative behavior usually requires kinship to function sustainably. *Homo sapiens* conduct “science” in “groups” to the apparent benefit of participants, despite their lack of genetic kinship. I observed and classified the grouping behavior of 12 *Homo sapiens* conducting science “projects.” Humans demonstrated wide variation in topic and project characteristics, but nonetheless tended to form groups to conduct projects. These findings support the continued research into science as a productive social activity.

*Keywords:* cooperation, groups, *Homo sapiens*, human, science

### INTRODUCTION

Cooperation provides benefits to groups of individuals by eliminating the costs of redundancy and competition. Cooperative behavior often relies upon kinship to persist and is unusual between non-related individuals across many taxa. Why and how cooperation can persist in groups of organisms without genetic relatedness is not well understood. Proposed explanations include reciprocity, social networking, or manipulation of conspecifics.

Humans (*Homo sapiens*) – a perplexing and contradictory study organism – exhibit social and antisocial behavior often without regard for genetic relatedness. Examples of divisive behaviors include “war” and “politics.” Differences in preferences and opinion can generate antisocial behavior. However, unrelated humans sometimes cooperate during activities. Perhaps the least explicable of these occasionally cooperative behaviors involves the observation of nature, testing of hypotheses, and composition of written abstracts, first described by Wolf (2009) as “science.” The frustrations and tribulations of this behavior discourages the performance of science by many humans.

My study investigated science performed by unrelated humans. Humans will occasionally perform science within intra-specific mutualisms known as “groups.” Although groups lack bonds of genetic kinship, cooperation may improve the quality of science and lower individual workloads. I tested the hypothesis that because of these benefits, humans prefer to conduct long-term and intensive science within groups.

### METHODS

I conducted my study between January 3 and March 8, 2018 over the course of a “foreign study program” (hereafter “FSP”) studying “biology,” the science of life. The FSP included 12 participants. Two leaders known as “teaching assistants” and 1 “professor” organized the FSP but for unknown reasons did not complete projects. Three distinct professors attended the FSP in succession, but professor identity did not appear to confound organization of groups. To preserve anonymity, each participant was given a 2 or 3-letter identification code, which did not in any way correspond with the initials of the participant. Each participant conducted 7 rounds of science, or “projects,” at 6 locations in Costa Rica and Little Cayman. This analysis excluded an additional project conducted by all participants on Little Cayman.

For each project, participants could freely associate to choose project topics and group membership at their discretion. After completion of the FSP, I classified each project into several descriptive categories. I counted the sizes of groups and group compositions of participants. I also conducted anthropological observations on activities unrelated to science but still performed by FSP participants.

### RESULTS

Participants completed a total of 31 projects. I observed significant variation in the characteristics of projects preferred by individual *H. sapiens* (Table 1).

Table 1. Notable extremes in characteristics of FSP science projects conducted by participants.

Individual(s)	Distinction	Number of projects	Mean number of projects by FSP participants
AMS, CEJ	Most feasible projects	6	4.4
MJ	Least feasible projects	2	4.4
AMS	Most projects with regular mealtimes	7	4.3
GSC, MJ	Fewest projects with regular mealtimes	2	4.3
MMP, PJV	Most projects which killed study organisms	5	3.1
AWC, AMS, BVH, CEJ	Most projects on charismatic megafauna	4	2.9
HIH	Fewest projects on charismatic megafauna	1	2.9

Only 3 projects (9.7%) were conducted by 1 participant; ABC, CEJ, and MMP each completed 1 solo project. All other projects included 2-4 participants. The average group size was 2.9 participants. GSC joined groups with the lowest average size (2.4 participants); BVH and MMP worked in groups with highest average size (3.4 participants). AEO and CRV had the peculiar honor of working in groups with an average size of exactly 3 participants.

Participants did not always conduct projects with all other participants. At the extremes, AWC conducted projects with all other FSP participants, while AMS did not work with 5 other participants. Participants did not work with an average of 2.67 other FSP participants. Two pairs of individuals worked together on 4 projects: CEJ/MMP and HIH/MJ.

The non-science activities performed by individuals over the course of the FSP, which occurred in groups and individually, included verbal and physical communication, sleeping, hiking, eating, writing, swimming, snorkeling, diving, personal hygiene, and karaoke. Informal observations also indicated that groups could divide responsibilities in projects such as writing and data collection; groups also had “fun” during the conduct of science.

## DISCUSSION

Over the course of the FSP, some individuals preferred feasibility, regular mealtimes, or studied charismatic megafauna, among other characteristics, while others exhibited exact opposite preferences. Nevertheless, groups rather than individuals conducted most of the projects on the FSP. This aggregation pattern could be explained by the division of labor and the “fun” experienced during group projects.

The prevalence of groups in the conduct of projects indicates the usefulness of science to promote social cooperation among humans. The variety of non-science activities performed by participants also solidified cooperative social behavior. Despite having no genetic relatedness, participants on this FSP achieved astonishing scientific and educational productivity. I propose entry into the scientific literature of the term “FSP family” to describe this phenomenon.

## ACKNOWLEDGMENTS

I thank all my friends on FSP for this amazing term, as well as Michael Brown, Debora Goedert, Matt Ayres, Celia Chen, and Mark Laidre for their instruction and kind advice.

## LITERATURE CITED

Wolf, R. D. D. 2009. Primate behavior: a study of our studies. *Dartmouth Studies in Tropical Ecology* 2009, pp. 256-259.



# IF OUR PROJECTS WERE HONEST: A REALISTIC HOT MESS IN *HOMO SAPIENS*

CORINNE R. VIETORISZ

*Abstract:* Conducting an entire experiment and writing up a full scientific paper in four days comes with a host of challenges that are exacerbated by the fact that FSP students generally have no idea what they're doing. This study outlines what *actually* happens during an FSP project throughout fieldwork and the writing process. Though our results are usually utter biological nonsense, we have become excellent at improvising field methods and pulling an entire project out of our behinds in a surprisingly short time.

*Key words:* ineptitude, hot mess, perseverance, science?, statistically insignificant, struggle

## INTRODUCTION

Nature is outside and does many important theoretical things. In fact, there are lots of organisms that do these theoretical things. Look, we found studies showing this (Scientist that is probably dead by now 1962)! But no one has studied these cool theories in a random organism we found that *probably* exhibits these theoretical behaviors? Bear with me, we didn't know the organism did these things until this morning so we're just going to frame our entire study this way. Anyway, our organism is truly a phenomenal study system for this theoretical behavior.

All of our background information on this organism comes from this one guy who did a bunch of studies on it in the '70s, but he did them on the opposite side of the world in a different ecosystem (Old white guy 1971, Old white guy 1974, Old white guy 1979). However, no one else on this godforsaken planet seems to give a flying frick about this organism, so we have no other literature to go off of.

We hypothesize that the organism exhibits an interesting behavior when exposed to a variable we introduced in the last three hours of our study, based on literature we found this morning after we had already done the whole experiment (McScienceson et al. 2012). We hypothesize this because we had to scrap all four of our previous hypotheses when we changed our experiment in the last three hours of our study. We predict that the organism **SHOULD** exhibit a certain behavior based on the literature, but we already know that they don't because we already did the experiment and literally just came up with the hypothesis.

## METHODS

### *Initial measurements*

We started out with a logical, simple sampling method that was definitely going to work. We planned on flawlessly executing the initial measurements but then our ruler broke. After duct-taping the ruler back together, we headed back out but then realized our study subject was realllly hard to reach. We then stared at our study subject in adamant confusion for exactly 21.5 minutes. After employing Herculean problem-solving skills, we then used more duct tape and taped our ruler to a stick so we could reach higher. After conducting seven measurements, we realized we had actually been measuring in inches instead of centimeters and had to go back and redo those measurements. Project member 1 called Project member 2 an idiot and Project member 1 hit them with the ruler-stick, causing the ruler to fall off the stick. We used the last few inches of duct tape and a hair tie to repair the tool. We then continued to take 20 more samples in almost complete silence, stopping only to manically slap 342 mosquitoes ranging in size from 0.5mm to 7mm.

### *More difficult measurements*

After taking the initial measurements, we realized our original plan was, in fact, just not possible because Nature (unbelievably) is not what we expected. We threw our data sheets into the fire and cried for 8.7 minutes. Upon consulting with the professor, we constructed an entirely new experiment and data collection design. Our spirits lifted, we headed back into the field only to realize the new collection design was infinitely more complicated, and we were out of duct tape. However, we could not make a fool in front of the professor to ask for

further help, so we gritted our teeth and commenced an utterly haphazard data collection. Project member 2 fell in a river and we had to wait for 21 minutes for her to run back and get dry clothes. Project member 1 laughed at project member 2 so she then pushed him into the same river. We subsequently had to wait for 28.3 minutes for project member 2 to run back and get dry clothes. Then it started pouring. Since our notebooks were not waterproof, we had to stop data collection and settle with  $N = 13$ , even though we said earlier we were definitely going to collect *at least* 20 samples.

### Statistical methods

We just wanted to run a linear regression, but upon consulting with the TAs we realized our data had 1,032 confounding factors that had to be accounted for in a complicated model. After our TA explained to us for 45 minutes how to run the model, we all convincingly nodded in understanding and then proceeded to run the model entirely incorrectly. Since none of us understood what the model actually does, we just BS'ed a description of what we think the model probably did. Then we realized we had never actually checked for normality and found out our data wasn't even normal so we "technically" shouldn't have run the model in the first place. On the bright side, at least we didn't have to run a PCA.

### RESULTS

We measured 13 organisms that ranged from too small to measure accurately to too large to measure with just a 30cm ruler taped to a stick (mean  $\pm$  SE = 20cm  $\pm$  25cm). We found these organisms in a variety of habitats, none of which matched where the literature said they should be.

Our supposedly meaningful predictor, in fact, did not predict the questionably accurate response variable, and the plot of the data looked like someone had eaten a bunch of data points and then painfully vomited them back up onto a set of axes (Figure 1; slope  $\pm$  SE = 0.11  $\pm$  0.31,  $P = 0.73$ ,  $r^2 = 0.01$ ). However, if we could switch the  $P$ -value and  $r^2$ , then our result would be really significant.

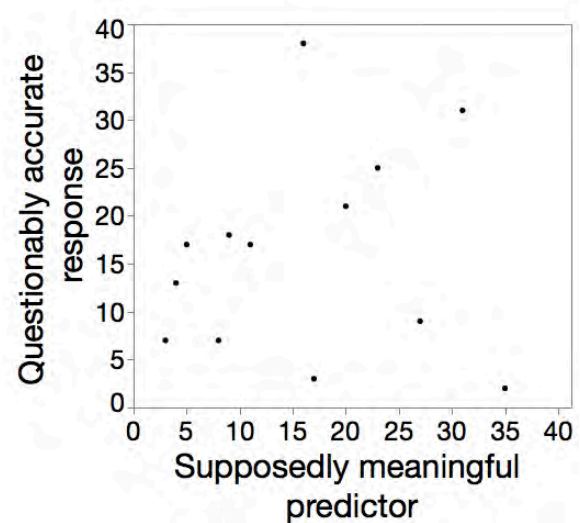


Figure 1. A really sad scatter plot with no correlation. Each point represents one sample. If you squint really hard, it kind of looks like a worm wearing a top hat.

Subsequent analyses of a bar graph proved to be more useless than our current government (Figure 2). I guess we could've run a t-test on it, but it doesn't take a PhD to see the area of overlap between the error bars is larger than Russia.

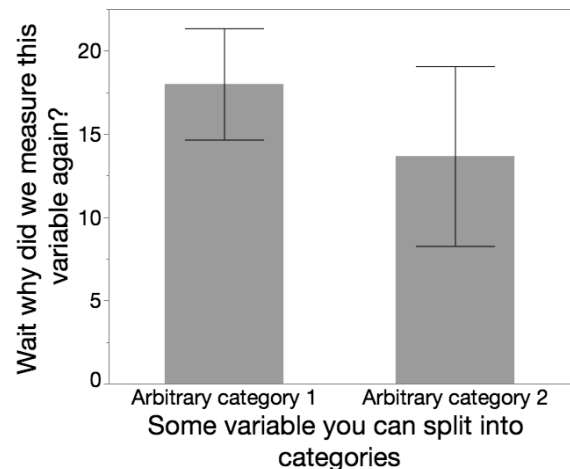


Figure 2. A bar graph with error bars so large it's honestly embarrassing. Error bars represent one standard error from the mean. If it were worth it to explain the graph, I would, but we honestly just measured this variable for sh\*t's and giggles.

### DISCUSSION

Overall, our project was truly a hot mess and I'm really surprised we were able to BS our whole paper thus far. While our results undoubtedly hold no biological significance, our

group learned many important things over the course of this project.

Firstly, all members of our group have fostered a newfound hatred for our study organism. Our results indicate that our organism doesn't do anything significant and is easily outmatched in intelligence by a rock. This organism provides evidence against Darwin's theory of survival of the fittest (Darwin 1859), because we have no clue how this species has made it through billions of years of evolution without immediately being naturally selected out of the gene pool. We would not be upset if this organism went extinct and would in fact encourage the selection process in this direction.

Furthermore, we gained extensive knowledge of the difficulty of *Homo sapiens* group work. Previous literature stated that I was a lovely personality and quite charming in social situations (My mom 2016); however, our findings completely contradict such notions. Over the course of the project, my irritability increased linearly as time since last meal increased. The likelihood of project members 1 and 2 erupting into an argument increased exponentially as the scientific feasibility of our project decreased.

Lastly, we were incredibly impressed with our own abilities to fool others into thinking we knew what we were doing. Never before have I used the words "elucidate," "indicate," "evaluate," "spatial," "temporal," "confounding," "subsequently," or "insight" with such frequency. Our ability to use fancy

language and convincing speech patterns to make us seem competent and polished to cover up our ineptitude are true indicators of our status as ivy-league college students.

In summation, we honestly tried to do science, and whatever we found can probably be linked to conservation.

#### ACKNOWLEDGEMENTS

We would like to thank the teaching assistants and our professor for essentially rewriting the whole paper for us.

#### AUTHOR CONTRIBUTIONS

All authors contributed equally, except Project Member 3 did more than her fair share of work mediating inane arguments between Project Members 1 and 2.

#### LITERATURE CITED

- Darwin, C. 1859. On the Origins of Species. Probably published by Darwin himself.
- McScienceson, P.D., G.F. Ecologowski, L.T. vonBiology. 2012. Examining the coolness of science in a fun ecosystem. *Neature*. 43:1125-1132.
- My mom. 2016. Dissertation on why I am a good child. *Behavioral mom-ology*. 32:679-683.
- Old white guy. 1971. Basic ecology of an organism. *Obscure journal*. 2:34-42.
- Old white guy. 1974. More basic ecology of the same organism. *Obscure journal*. 11:56-61.
- Old white guy. 1979. Still the same organism but this time with an experiment. *Obscure journal*. 23:46-53.

# DECLINES IN THE CHARISMA OF TROPICAL TAXA

BALTHASAR L. VON HOYNINGEN HUENE

**Abstract:** Tropical ecosystems are home to a wide variety of charismatic species. However, there is evidence for a decline in the perceived charisma of well-studied species among researchers (Victorisz 2018). We examined how the charisma, or “likeability,” of these species changes over a period of study and found that researchers’ opinions of their study species drop precipitously over time.

**Key words:** Charismatic species, Neotropics, frustration, damn animals, damnnimals, Millennials, hatred, useless good-for-nothing species, extermination

## INTRODUCTION

The Neotropics are home to some of the most biodiverse regions on the planet. The abundance and diversity of organisms, including many charismatic taxa such as large mammals, attract both tourists and researchers alike. However, recent literature suggests that the charismatic nature of some species tends to evaporate in the opinions of researchers studying those species, leading to hostility, “newfound hatred,” and anti-conservationist attitudes (Victorisz 2018). We investigated the severity of this shift in attitude among researchers.

## METHODS

We focused on a cohort of undergraduate researchers studying a diverse array of taxa in tropical ecosystems. In order to assess how their opinions of their chosen study species changed over time, we simply remained within earshot towards the beginning and end of their projects. We assessed the perceived charisma of their study species by qualitative analysis of their nonstop complaints, vents of frustration, and howls of unrestrained rage over the intractable behavior and biology of those species.

## RESULTS

Researchers’ initial attitudes toward their study species were characterized by enthusiasm and fascination, showing that these species were viewed as charismatic. (This was even true for some ectothermic species that the general public would regard as hideous, revolting, and certainly to be avoided at all hazards.) When confronted with the task of quantifying a pattern in the biology of such a species, the researchers tempered their exuberance, which frequently

yielded to a dark and deep-seated loathing (Table 1).

Table 1. Changes in researchers’ attitudes over study period by taxonomic group.

TAXON	ATTITUDE AT BEGINNING OF STUDY	ATTITUDE AT END OF STUDY
Agouti ( <i>Dasyprocta sp.</i> )	“The agouti is an impressive opportunistic forager, able to exploit a wide range of food sources to maximize its caloric intake.”	“These things are just creepy, oversized rats. I used to think they were cute, but the way they stare at me gives me the heebiejeebies.”
Scarlet Macaw ( <i>Ara macao</i> )	“Macaws are not only an iconic tropical megafauna, but some of the most intelligent and behaviorally complex avians.”	“They hate us and want us to fail.”
Bromeliads ( <i>Bromeliaceae</i> )	“The microbiomes within bromeliads are some of the most unique and fascinating examples of plant-animal symbiosis to	“Good Lord, these plants are boring. And inaccessible. And the critters in their water reservoirs are disgusting. I

	be found anywhere.”	can’t believe I was talked into another plant project.”
Stick insects ( <i>Phasmatodea</i> )	“Stick-mimicking phasmids are a striking example of visual crypsis, a trait that has evolved independently in many branches of the phylogenetic tree.”	“Whose genius idea was it to study a bug whose survival hinges on the fact that NOTHING CAN FIND IT? Even if I could find one, I’d probably feed it to a bird out of spite.”
Long-spined sea urchin ( <i>Diadema antillarum</i> )	“The long-spined sea urchin is crucial to maintaining the health of an intact coral reef, preventing algal overgrowth by constant grazing.”	“Giant malicious pincushions. Data collection was a series of near-death experiences. I have nightmares about them now.”

## DISCUSSION

This decline in perceived charisma of tropical taxa can probably be attributed to the disconnects between the researchers’ theoretical, *a priori* expectations and the quantifiable aspects of tropical ecology. Those disconnects

result in psychological trauma upon the realization that, heck, I don’t have a CLUE how this amphibian responds to predation threats, nor how the growth of that plant varies by proximity to canopy gaps, nor how the schooling behavior of that fish I saw on the first day at this site (and ONLY on the first day) varies by channel depth, and frankly if I DID have a clue it would take me a whole WEEK of head-scratching and navel-gazing to figure out how to measure it, and I’m fine with that EXCEPT that if I don’t have data in hand before DINNER tonight I’ll be up the proverbial academic CREEK without a PADDLE, and I don’t CARE if it isn’t that species’ fault that I don’t know how to study it, I’m SUFFERING and it’s all because of those DAMN FROGS and I wish they would all DISAPPEAR like those hymenopterans did in the middle of my data collection last week.

Our findings can probably be extrapolated to systems beyond neotropical species. For example, in this particular investigation, our study system was a group of Millennial undergraduates. Our opinion of Millennial undergraduates was steadily eroded throughout the course of our research, consistent with the pattern described in our results. However, this trend may have been confounded by sleep deprivation, hostile climatic conditions, and nutritional irregularities, and should probably be investigated further.

## LITERATURE CITED

Vietorisz, C. 2018. If our projects were honest: A realistic hot mess in *Homo sapiens*. Dartmouth Studies in Tropical Ecology (2018).