

MEMBERS OF THE DARTMOUTH BIOLOGY FSP 2012

FACULTY

RYAN G. CALSBEEK MATTHEW P. AYRES CELIA Y. CHEN

LAB COORDINATOR

CRAIG D. LAYNE

GRADUATE ASSISTANTS

MICHAEL L. LOGAN NINA K. LANY SAMUEL B. FEY

UNDERGRADUATES

WALES A. CARTER
M. HEMAYAT R. CHOWDHURY
ROBIN A. COSTELLO
ANNA L. DEFFEBACH
SHEA E. FLANAGAN
NINA B. FRANKEL
MADILYN M. GAMBLE

MILO S. JOHNSON
BENJAMIN J. KESSLER
MADELINE K. KREHER
JAMIE E. McLAUGHLIN
JESSE T. RIEB
AMY E. VAN SCOYOC

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

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

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

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

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

We acknowledge the generous financial support of Dorothy Hobbs Kroenlein.

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

Matthew P. Ayres
Professor of Biological Sciences and Director of Biology Foreign Studies Program
Life Sciences Center, 78 College Street
Dartmouth College
Hanover, NH 03755



603 646-2788 lab
603 359-7231 cellphone
<http://www.dartmouth.edu/~mpayres/>
Matt.Ayres@Dartmouth.Edu

Bio Foreign Studies Program: schedule for Costa Rica 2012.					
Date	Day	Location	Morning	Afternoon	Evening
5-Jan	Wed	To San Jose	Travel	Travel	Arrive in evening
6-Jan		In San Jose	OTS, InBIO	free: shopping etc.	Group dinner in SJ
7-Jan		To Palo Verde	Travel	Orientation	Lec: Intro CR ecol (RC, HC)
8-Jan	Sun	At PV	Orientation	Lec: Primate Ecol (ML, AD). Res. Qs.	Lec: Avian Ecol (NL, NF)
9-Jan		At PV	FP-1 (ant-acacia)	Stat lab (ML/NL)	Data analysis/synthesis
10-Jan		At PV	FP-2	ArthLab(NL/RC). Lec: Behav (RC, WC)	FP-1 symposium. Writing.
11-Jan		At PV	FP-2	VertLab(RC/ML). Lec: DivCoex (RC, JR)	Writing. FP-1 ms due
12-Jan		At PV	SIFP-1 plan/proposals	Plant lab (NL)-Data Anal/Writ.	FP-2 seminars. Writing
13-Jan		At PV	SIFP-1	SIFP-1	Writing. FP-2 ms due.
14-Jan		At PV	SIFP-1	SIFP-1/analysis. Revisions.	SIFP-1/anal. Revs.
15-Jan	Sun	At PV	River trip	SIFP-1 symposium. Writing.	Writing: SIFP-1 ms due
16-Jan		To Santa Rosa	Travel/walk	Orientation. Lec:Turtles(ML)	Field: Sea turtle nesting
17-Jan		At SR	Lec: Mngrv(NL)	Exploration	Field: Sea turtle nesting
18-Jan		To Monteverde	Travel	Orientation	Writing (revisions)
19-Jan		At MV	Orientation	SIFP-2 planning	Lec: Amphibs? (A Pounds?)
20-Jan		At MV	SIFP-2 pilot /props	SIFP-2	Lec: His/Orig (RC, MK)
21-Jan		At MV	SIFP-2	SIFP-2	Analysis.Writing (rev.)
22-Jan	Sun	At MV	SIFP-2	Analysis/synthesis	SIFP-2 symposium
23-Jan		At MV	Writing	Writing SIFP-2 ms due. Bat Ingl	Writing
24-Jan		At MV	All final mss due	Exploration	Free
25-Jan		To Cuerici	Travel	Travel/Orientation	Lec: Coevol 1 (MA, SF)
26-Jan		At Cuerici	Trip to Paramo	Orientation	Writing lab 1 (MA)
27-Jan		At Cuerici	SIFP-3 planning / proposals	SIFP-3 pilot	SIFP-3 final proposals
28-Jan		At Cuerici	SIFP-3	SIFP-3	Lec: Coevol 2 (MA, BK)
29-Jan	Sun	At Cuerici	SIFP-3	SIFP-3	Analysis, writing
30-Jan		At Cuerici	Analysis, writing	SIFP-3 symposium	Writing SIFP-3 ms due
31-Jan		To La Palma	Exploration at Cuerici	Travel to La Palma	Sirena preparation
1-Feb		To Sirena	Hike to Sirena	Hike to Sirena	Natural history reports from hike
2-Feb		At Sirena	Orientation	SIFP-4 plan	Lec: Social insects (MA, AvS)
3-Feb		At Sirena	SIFP-4 pilot	SIFP-4	Lec: Plant-Herb. interactions (NL, MG)
4-Feb		At Sirena	SIFP-4	SIFP-4	Writing lab 2 (MA)
5-Feb	Sun	At Sirena	SIFP-4	SIFP-4	Analysis. Writing
6-Feb		At Sirena	SIFP-4	Analysis. Writing	SIFP-4 symposium.
7-Feb		To Las Cruces	Hike out of Sirena	Travel to Las Cruces	Writing
8-Feb		At Las Cruces	Orientation	Writing. Botany.	Writing
9-Feb		At Las Cruces	Writing. Botany.	Writing. Botany practicum.	What is the purpose of science?
10-Feb		To La Selva	Travel	Travel	Lec: Aquatic Ecology (SF, MJ)
11-Feb		At La Selva	Orientation	SIFP-5 plan/pilot	SIFP-5 proposals. Night walk.
12-Feb	Sun	At La Selva	SIFP-5	SIFP-5	Lec: Herpetology (ML, RC)
13-Feb		At La Selva	SIFP-5	SIFP-5	Lec: Ecosystems (SF, JM)
14-Feb		At La Selva	SIFP-5	SIFP-5	Lec: Cons. Biology (MA)
15-Feb		At La Selva	SIFP-5	Analysis. Writing	Analysis. Writing. Night walk.
16-Feb		At La Selva	Agroecology field trip	Analysis. Writing	SIFP-5 symposium.
17-Feb		At La Selva	Final revisions to all CR mss.	Final revisions to all CR mss.	Final revisions to all CR mss.
18-Feb		To San Jose	Exploration	Travel to SJ	Free
19-Feb	Sun	To Little Cayman until 8 March			
FP = "Field problem" (staff initiated)					
SIFP = "Student initiated field problems".					
Each FP and SIFP culminates in a seminar presentation and a polished manuscript for publication in <u>Dartmouth Studies in Tropical Ecology</u> .					

Schedule for Little Cayman 2012

The schedule is very likely to change depending on weather conditions, which influence when we are able to dive. When the weather is good (low winds), we will do a dive. See www.windfinder.com, winds less than 10 knots are best but we can dive when winds are slightly greater (15) but from the east or southeast. Nearly all morning and afternoon activities will be outdoors and will usually last from 8-12 and 1-5. Take 1 Bonine or Dramamine the NIGHT BEFORE a dive and also in the morning depending on your susceptibility to sea sickness.

Breakfast is at 7 am, lunch at noon, and dinner at 6 pm. If you will be late for lunch or dinner, then ask someone to set food aside.

IF YOU GO ANYWHERE, ALWAYS GO WITH A BUDDY AND SIGN OUT ON THE WHITEBOARD IN THE DINING HALL.
Enjoy these 3 weeks in paradise!

Date		Morning	Afternoon	Evening
19 Feb Sun	Arrive from CR			<u>13 people arrive 5:35 pm</u> Unpack Main orientation and safety information – Rob, Celia, and Perry
20 Feb Mon	Orientation	Discussion about program to date and expectations for LC segment. Unpack equipment Get BCD and regulator from Lowell <i>Assign expert taxonomic groups</i>	Coral biology lecture & Reef morphology (CC) Snorkel to see coral followed by <i>Natural history discussion before dinner</i>	Critique: Coral biogeochemistry (Robin Costello) and Coral Recruitment (Nina Frankel)
21 Feb Tues	General natural history	SCUBA –shore dive at Cumber’s Cave (check dive)	Algae lecture and Sea Grass lecture & lab (CC) Snorkel to see invertebrates and collect algae followed by <i>Natural history discussion before dinner</i>	Critique: Coral/Algae interaction (Menayat Chowdhury) and Invertebrate Ecology (Maddie Gamble)
22 Feb Wed	General natural history	SCUBA – Sailfin and Meadows	Zooplankton and Invertebrate lecture (CC) Project 1 exploration <i>Natural history discussion before dinner</i>	Critique: Invertebrate ecology II (Milo Johnson) Zooplankton lab (SF) & night sampling
23 Feb Thurs	Project 1 begins	Fish ecology (CC) Snorkel to see sponge lab (ML)	3:30pm – Finalize project 1 idea, design, and group members 4:30 – Fish behavior lecture (ML) Weekly Group Meeting	Critique: Fish larvae recruitment (Shea Flanagan) and Diversity and Coexistence (Carter Wales) Project 1 idea discussion

Date		Morning	Afternoon	Evening
24 Feb Fri	Project 1 proposal	Project 1 - pilot	Project 1	Fish Behavior (Anna Deffebach) RR: Karaoke for those interested
25 Feb Sat	Dia libre (OFF)		Beach Cleanup	BBQ
26 Feb Sun	Project 1 proposal DUE	Project 1	Project 1	Critique: Trophic Cascades I (Benji Kessler) and II (Madeline Kreher)
27 Feb Mon	Project 1	Project 1	Coral reefs and climate change (CC) Project 1	Critique: Coral Reef decline (Jamie McLaughlin) and II (Jesse Rieb)
28 Feb Tues	Project 1	SCUBA – Marilyn’s Cut & Soto Trader Project 1	Tropical Invasives (SF) Project 1 - writing and analysis	Project 1 – data analysis, write methods, stats help
29 Feb Wed	**Project 1 DUE** Project 2 begins	Project 2 brainstorming & pilot	Conservation and Management (CC) Project 2 1pm - Project 2 idea discussion	**Project 1 PRESENTATIONS**
1 Mar Thurs	Project 2 proposal DUE	Project 2	Project 2	Critique: Fish Community Response (Amy van Scoyoc) Finalize project 2 idea, design, and group members Graduate school discussion Grouper Moon video
2 Mar Fri		SCUBA - Coconut Walk & Sara’s Set	Project 2 Weekly Group Meeting	RR: Karaoke for those interested
3 Mar Sat	Project 2	Project 2	Project 2	Night dive – Cumber’s Caves
4 Mar Sun	Project 2	Project 2	Project 2	**Project 2 – writing and analysis
5 Mar Mon	**Project 2 DUE @ 6pm**	Project 2	Project 2 – writing and analysis Presentations: Project 2	**Project 2 DUE @ 6pm** Clean up science equipment and field sites
6 Mar Tues		SCUBA – Baracuda Bight & Great Wall West Clean up	**Project 2 revisions DUE @ 1pm** Revisions and copy editing of all LC projects	Revisions & copy editing of all LC projects Discussion of papers on authorship
7 Mar Wed		Revisions & copy editing of all LC projects	Clean up & pack	Dinner and campfire (pending wind)
8 Mar Thurs	Depart LC 9:00 am			

PAPERS FOR PRESENTATION BY STUDENTS TO THE GROUP WHILE IN COSTA RICA.

Student	Student Paper
Hemayat R. Chowdhury	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.)
Nina B. Frankel	Emlen, S. T. and P. H. Wrege. 2004. Size dimorphism, intrasexual competition, and sexual selection in wattled jacana (<i>Jacana jacana</i>), a sex-role-reversed shorebird in Panama. <i>The Auk</i> 121(2): 391-403.
Anna L. Deffebach	Fedigan, L. M. and Jack, K. M. 2011. Two girls for every boy: the effects of group size and composition on the reproductive success of male and female white-faced capuchins. <i>American Journal of Physical Anthropology</i> 144:317-326.
Wales A. Carter	Irwin D.E. et al. 2001. Speciation in a ring. <i>Nature</i> 409, 333-337.
Jesse T. Rieb	Molino, J.-F. and D. Sabatier. 2001. Tree diversity in tropical rain forests: a validation of the intermediate disturbance hypothesis. <i>Science</i> 294:1702-1704.
Madeline K. Kreher	Janzen, D. H. 1981. Neotropical anachronisms: the fruits the gomphotheres ate. <i>Science</i> 215:19-27.
Shea E. Flanagan	Ramirez, S. R., T. Eltz, M. K. Fujiwara, G. Gerlach, B. Goldman-Huertas, N. D. Tsutsui, and N. E. Pierce. 2011. Asynchronous diversification in a specialized plant-pollinator mutualism. <i>Science</i> 333:1742-1746.
Benjamin J. Kessler	Becerra, J. X., K. Noge, and D. L. Venable. 2009. Macroevolutionary chemical escalation in an ancient plant-herbivore arms race. <i>Proceedings of the National Academy of Sciences of the United States of America</i> 106:18062-18066.
Amy E. Van Scoyoc	Waters, J. S., C. T. Holbrook, J. H. Fewell, and J. F. Harrison. 2010. Allometric scaling of metabolism, growth, and activity in whole colonies of the seed-harvester ant <i>Pogonomyrmex californicus</i> . <i>American Naturalist</i> 176:501-510.
Madilyn M. Gamble	Novotny, V. et al. 2006. Why are there so many species of herbivorous insects in tropical rainforests? <i>Science</i> 313: 115. (* see also: "Crafting the pieces of the diversity jigsaw puzzle" by R.L. Kitching from the same issue).
Robin A. Costello	Anchukaitisa, K. J. and Evansa, M. N. 2010. Tropical cloud forest climate variability and the demise of the Monteverde golden toad. <i>Proceedings of the National Academy of Sciences of the United States of America</i> 107:5036-5040.
Milo S. Johnson	Anderson, J. T., T. Nuttle, J. S. S. Rojas, T. H. Pendergast, and A. S. Flecker. 2011. Extremely long-distance seed dispersal by an overfished Amazonian frugivore. <i>Proceedings of the Royal Society B-Biological Sciences</i> 278:3329-3335.
Jamie E. McLaughlin	Yvon-Durocher, G., J. I. Jones, M. Trimmer, G. Woodward, and J. M. Montoya. 2010. Warming alters the metabolic balance of ecosystems. <i>Philosophical Transactions of the Royal Society B-Biological Sciences</i> 365:2117-2126.

PAPER PRESENTATIONS FOR LITTLE CAYMAN

Coral biology - biogeochemistry (Robin Costello)

Stat et al. 2008. Functional diversity in coral-dinoflagellate symbiosis. *PNAS* 105: 9256.

Coral/Algae interaction (Hemayat Chowdhury)

Rasher et al. 2011. Macroalgal terpenes function as allelopathic agents against reef corals. *PNAS* 108: 17726.

Fish Behavior (Anna Deffebach)

Meyer, J. L. and E. T. Schultz. 1985. Migrating haemulid fishes as a source of nutrients and organic matter on coral reefs. *Limnology and Oceanography* 30:146-156.

Fish larvae recruitment (Shea Flanagan)

Allmany, G.R. 2007. Local replenishment of coral reef fish populations in a marine reserve. *Science* 316: 742-744.

Coral Recruitment (Nina Frankel)

Albright et al. 2010. Acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. *PNAS* 107: 20400.

Invertebrate ecology I (Madilyn Gamble)

Przeslawski R, Anyong S, Byrne M, Worheide G, and Hutchings P. 2008. Beyond corals and fish: the effects of climate change on noncoral benthic invertebrates of tropical reefs. *Global Change Biology* 14: 2773–2795.

Invertebrate ecology - Zooplankton (Milo Johnson)

Ohlhorst, S.L., *Diel migration patterns of demersal reef zooplankton*. *Journal of Experimental Marine Biology and Ecology*, 1982. **60**(1): p. 1-15.

Diversity and coexistence (Wales Carter)

Munday, P. L. (2004). Competitive coexistence of coral-dwelling fishes: The lottery hypothesis revisited. *Ecology* 85(3): 623-628.

Trophic cascades I (Benjamin Kessler)

Mumby, P.J. et al. 2007. Trophic cascade facilitates coral recruitment in a marine preserve. *PNAS* 104: 8362-8367.

Trophic cascades II (Madeline Kreher)

Mumby, P.J. et al. 2006. Fishing, trophic cascades and the process of grazing on coral reefs. *Science* 311: 98-101.

Coral reef decline I (Jamie McLaughlin)

Lesser, M.P. 2007. Coral reef bleaching and global climate change: Can corals survive the next century? *PNAS* 104:5259-5260.

Coral reef decline II (Jesse Rieb)

Pandolfi, J.M. and J.B.C. Jackson. 2006. Ecological persistence interrupted in Caribbean coral reefs. *Ecology Letters* 9: 818-826.

Fish community response to coral decline (Amy van Scoyoc)

Jones, G.P, M.I. McCormick, M. Srinivasan and J.V. Eagle. 2004. Coral decline threatens fish diversity in marine reserves. *PNAS* 101:8251-8253.

REQUIRED READINGS FOR LITTLE CAYMAN

Hoegh-Guldberg et al. 2007. Coral reefs under rapid climate change and ocean acidification. *Science* 318: 1737.

Hughes et al. 2010. Rising to the challenge of sustaining coral reef resilience. *TREE* 25: 633.

Odum and Odum. 1955. Structure and productivity of a windward coral reef community on Eniwetok Atoll. *Ecological Monographs* 25:291.

Hixon. 2011. 60 years of coral reef fish ecology: past, present, future. *Bull. of Marine Science* 87: 727.

Nybakken and Bertness. 2005. *Marine Biology: An Ecological Approach*. 6th Edition, Pearson Education Inc. Chapter 9: Coral Reefs, pp. 407-453.

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ANT DENSITY AND INDIVIDUAL AGGRESSION HAVE DIFFERENT EFFECTS ON THE RESPONSE TO SIMULATED HERBIVORY AMONG THREE SPECIES OF ACACIA-ANT

Wales A. Carter, Robin A. Costello, Madilyn M. Gamble, Benjamin J. Kessler, Jamie E. McLaughlin

Project Design: Michael L. Logan. Faculty Editor: Ryan Calsbeek

Abstract: Although the mutualism between Acacia trees and ants in the New World Tropics is well-studied, interspecific variation in anti-herbivory behavior remains largely unexplored. We investigated the rate of response to simulated herbivory and its dependence on initial ant density in three species of Acacia ant, *Pseudomyrmex flavicornis*, *Pseudomyrmex spinicola*, and *Crematogaster brevispinosa*. *P. spinicola* ants are known to be the most aggressive on the individual level. We hypothesized that *P. flavicornis* and *C. brevispinosa* would compensate for lower individual aggressiveness by either responding faster or in greater numbers to a herbivory event. For all three species, ant activity peaked within the first fifteen seconds following disturbance by a simulated herbivore. We found that the relationship between pre-herbivory ant density (PHAD) and the percent change in activity following a simulated herbivory event varied among species. Our results suggest that some species compensate at the colony level for lower individual aggressiveness, though other species do not.

Keywords: *Acacia*, *Pseudomyrmex flavicornis*, *Pseudomyrmex spinicola*, *Crematogaster brevispinosa*, *herbivory*, *mutualism*, *ant communication*

INTRODUCTION

Mutualisms between plants and animals greatly increase the success of their participant species in many ecosystems. Several species of ant, including *Pseudomyrmex flavicornis*, *Pseudomyrmex spinicola*, and *Crematogaster brevispinosa*, form complex obligate mutualisms with the acacia tree *Acacia collinsii*. Acacia trees provide food resources and shelter for the colony while the ant colony defends the tree from competition with neighboring plants and from herbivorous insects and mammals (Janzen et al. 1973). Ants protect the trees from herbivory and other disturbances with a localized defensive response coordinated by pheromones (Janzen 1983). *Pseudomyrmex spinicola* is known to be more aggressive on an individual level than both of the other acacia ant mutualists, in that individual *P. spinicola* ants win one-to-one combat encounters with other ant species (Aho et al 2011). In this paper, we will refer to this pattern as “individual aggressiveness”. We assume that ants that are more aggressive towards other ants are also more aggressive

towards herbivores.

We assumed that less individually aggressive species would be at a defensive disadvantage and therefore would have to compensate by either responding faster to herbivory or by mounting a larger numeric response. The goal of this study was to better understand (1) interspecific variation in the timing of these defensive responses and (2) how these varied in magnitude as a function of pre-herbivory ant density (PHAD) across different levels of individual ant aggression. We expected less individually aggressive ants to respond faster than more individually aggressive ant species to a simulated herbivory event. Furthermore, we hypothesized that the more individually aggressive *P. spinicola* would need fewer ants to defend the tree and therefore a greater PHAD would result in a less intense response. Alternatively, we expect the less individually aggressive *P. flavicornis* and *C. brevispinosa* to mount a larger response regardless of PHAD. We tested these hypotheses by disturbing acacia trees inhabited by each of these ant species and measuring changes in ant activity after the

simulated herbivory event.

METHODS

We conducted our study between the hours of 0730 and 1130 on 9, January, 2012 in a large stand of Ant-Acacia trees (*Acacia collinsii*) in the tropical dry forest of Palo Verde National Park, Costa Rica (10.352°N, 85.308°W). We haphazardly selected trees housing one of three ant species: *Pseudomyrmex spinicola*, *Pseudomyrmex flavicornis*, or *C. brevispinosa*. All trees were within 10 meters of the road. Our sample consisted of 15 trees associated with *P. spinicola*, 14 with *C. brevispinosa*, and 8 with *P. flavicornis*. On each tree we selected a 10cm section of the trunk at approximately 1.8 meters above the ground as our focal area for observation. Each focal area contained at least one domatia, the horn-like growths acacia trees provide to house their ant mutualists. For one minute we recorded the cumulative number of ants that crossed an imaginary line drawn through the center of the focal area. We did not mark individual ants, and every ant crossing the line was considered an independent event. We defined this count as “activity.” We also recorded the total number of ants within the focal area at the beginning of each 15-second interval and defined this number as “density.” In the same 180° focal area, one observer recorded activity and one observer recorded density. After estimating the initial density and activity of ants within the focal area, we simulated an herbivory event by delivering a single hard blow with a 2cm diameter stick to the center of each focal area. We then estimated ‘post-herbivory’ density and activity in exactly the same way as described above.

We used a Repeated Measures ANOVA comparing mean pre-herbivory activity for each tree to its subsequent post-herbivory activity at each 15-second interval to examine the timing of peak response by each species. We also compared intervals containing the

greatest changes in activity with paired t-tests. To test the effect of initial density on the change in post-herbivory activity among species, we examined the correlation between mean initial density (averaged over one minute) with peak percent change in activity following simulated herbivory using ANCOVA. We repeated this analysis twice, including and excluding six significant statistical outliers. After we removed statistical outliers, our sample consisted of 13 trees housing *P. spinicola*, 9 housing *C. brevispinosa*, and 8 housing *P. flavicornis*. We conducted statistical analyses and removed statistical (boxplot) outliers using the statistical package JMP 9 (SAS Institute, Cary, NC).

RESULTS

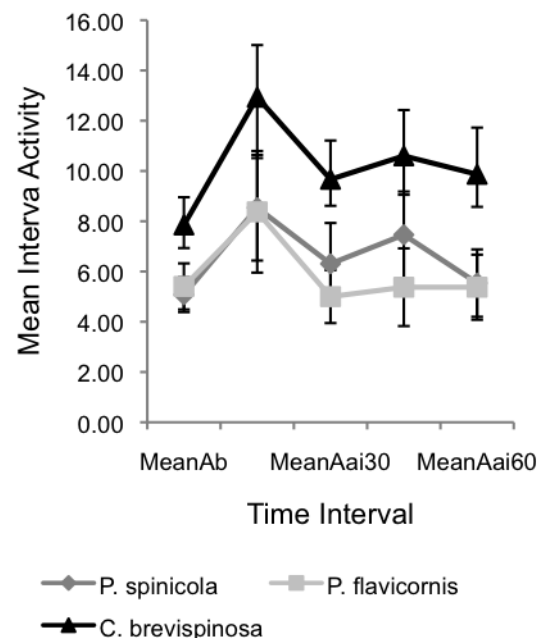


FIG 1. Activity of three species of Acacia ant before a simulated herbivory event and during the first four 15-second intervals after a simulated herbivory event. Ant activity significantly increased during the first 15 second interval in *P. spinicola* (2-tailed Matched Pairs t-test: $t = 3.61$, $df=13$, $P=0.003$), and we observed similar non-significant trends in *P. flavicornis* and *C. brevispinosa*. We also observed the greatest decrease in activity in the next 15 second interval (15 to 30 seconds), although these trends were not significant. Error bars show 1 SE.

Considering all trees together, we found a significant effect of time interval on the total ant activity following a simulated herbivory event (Repeated Measures ANOVA $F_{4,30}=4.16$, $P=0.009$; Fig.1). We used two-sided matched pairs t-tests to compare individual time intervals and found that maximum ant activity occurred within the first 15 seconds following a simulated herbivory event in *P. spinicola* ($t = 3.61$, $df=13$, $P=0.003$). Similar, but non-significant trends occurred in *P. flavicornis* and *C. brevispinosa* (*P. flavicornis*: $t = 1.93$, $df=7$, $P= 0.095$, *C. brevispinosa*: $t =1.87$, $df=8$, $P=0.098$) We observed that the greatest decrease in activity occurred between 15 and 30 seconds post-herbivory, but this trend was not significant for any of the species we tested (*P. spinicola*: $t=-1.89$, $df=13$, $P=0.081$, *P. flavicornis*: $t=-2.14$, $df=7$, $P=0.07$, *C. brevispinosa*: $t=-0.89$, $df=8$, $P=0.397$). By the end of our 1-minute observation period, we found that only *P. spinicola* retained an activity level significantly different from their average pre-herbivory activity ($t=2.28$, $df=13$, $P=0.04$).

TABLE 1. ANCOVA summary table of analysis of pre-herbivory density and species without statistical outliers.

Source	DF	Sum of Squares	F Ratio	Prob > F
MeanDb	1	0.12119	0.123	0.7280
Species	2	1.82059	0.930	0.4083
Species*MeanDb	2	6.70654	3.425	0.0491*

TABLE 2. ANCOVA summary table of analysis of pre-herbivory density and species with statistical outliers.

Source	DF	Sum of Squares	F Ratio	Prob > F
MeanDb	1	0.50700	0.137	0.7136
Species	2	3.04205	0.412	0.6660
Species*MeanDb	2	2.30042	0.311	0.7347

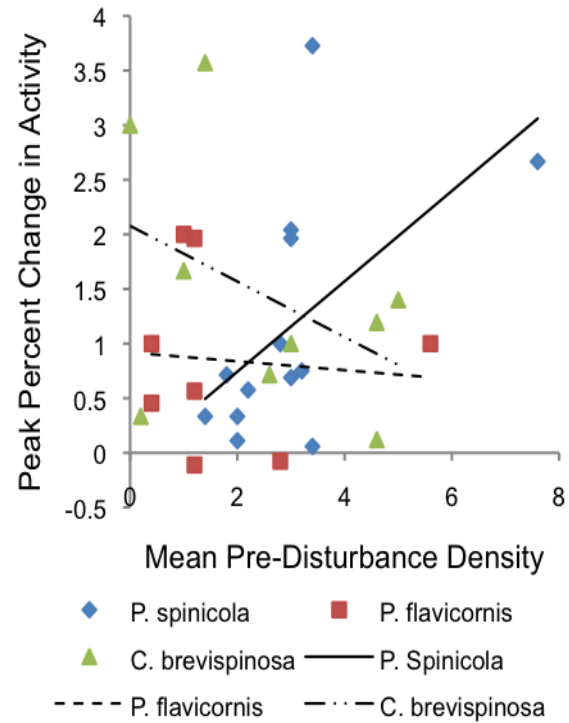


FIG 2. Relationship between pre-disturbance density and level of response to disturbance for three species of acacia-ant with significant outliers removed. Peak percent change in activity after simulated herbivory was significantly affected by initial density differently for three species of Acacia-ants. *P. spinicola* had an increased response to disturbance with a greater initial density, while *P. flavicornis* had similar responses regardless of prior density and *C. brevispinosa* had a decreased response with a greater pre-disturbance density.

We also observed that the relationship between initial ant density and percent change in activity after simulated herbivory varied among species. *P. spinicola* displayed an increased magnitude of response with a greater pre-herbivory density, *P. flavicornis* showed a similar response to a simulated herbivory event regardless of prior initial density, and *C. brevispinosa* displayed a decreased response with a greater pre-herbivory density (significant interaction between initial density and species when we removed significant outliers; ANCOVA effect test: $F_{2,30}=3.465$, $P=0.047$; table 1, fig. 2). When we retained statistical outliers in our analysis, there was no effect of either pre-herbivory density or species on the percent change in activity (ANCOVA effect test: $F_{2,35}=0.331$,

$P=0.73$; table 2).

DISCUSSION

Our results indicate an immediate, qualitatively identical peak in response rate to a simulated herbivory event for all three acacia-ant species. This result does not support our hypothesis. The less individually aggressive *P. flavicornis* and *C. brevispinosa* did not compensate for their lower individual aggressiveness by responding to a disturbance faster. As all species exhibited a similarly rapid response to herbivory, it is possible that the individual aggression of an ant species is not correlated with the speed of response. Interestingly, *P. spinicola* was the only species we studied which retained an elevated activity level 1 minute after the herbivory event. While the speed of response was uniformly quick among species, the duration of the response lasted longer in the more individually aggressive ant species. The continued elevation of activity in *P. spinicola* may combine with its individual aggressiveness to ensure greater effectiveness in defending its host tree.

While all three acacia-ant species responded quickly to a simulated herbivory event, we found no significant interaction between the magnitude of their responses and their PHAD. However, these results were dominated by six statistical outliers. When these outliers were removed, the interaction became statistically significant. Contrary to our prediction, in relation to the other species (1) *Pseudomyrmex spinicola*'s response to an herbivory event increased with an increased PHAD (2) *Crematogaster brevispinosa*'s response to an herbivory event decreased with an increased PHAD, and (3) *Pseudomyrmex flavicornis*'s response did not vary with PHAD.

We interpret the result that *P. spinicola* increased its response with increased PHAD

as an emergent property of the colony. Because the individual *P. spinicola* is more aggressive, all ants respond more aggressively to simulated herbivory.

Of the two less individually aggressive species, *P. flavicornis* was the only species that followed our prediction. *Pseudomyrmex flavicornis* appears to compensate at the colony level for lack of individual aggression. However, we do not understand what might account for this difference. This result raises the question of how each species differs in its co-ordination of response on the colony level.

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PERSISTENCE OF *CREMATOGASTER BREVISPINOSA* IN THE PRESENCE OF AN AGGRESSIVE RIVAL

M. HEMAYAT R. CHOWDHURY, MADELINE K. KREHER, JESSE T. RIEB, AND AMY E. VAN SCOYOC

Project Design: Nina K. Lany. Faculty Editor: Ryan Calsbeek

Abstract: According to the competitive exclusion principle, two species cannot coexist while subsisting on the same resource. *Crematogaster brevispinosa* and *Pseudomyrmex spinicola* are two ant species that exhibit a mutualism with the tree *Acacia collinsii*. In apparent contradiction of the competitive exclusion principle, both species persist while competing for the nectar that is provided by *A. collinsii*. We investigated two potential characteristics of *C. brevispinosa* that would allow it to compete with the more aggressive *P. spinicola*: 1) a greater ability to colonize young *Acacia collinsii* and 2) a greater rate of reproduction. Whereas dispersal ability did not differ between ant species, *C. brevispinosa* produced more larvae per cm³ than *P. spinicola*. We conclude that this reproductive advantage may allow *C. brevispinosa* to persist alongside its more aggressive competitor.

Key words: *Acacia collinsii*, coexistence, competition, *Crematogaster brevispinosa*, *Pseudomyrmex spinicola*

INTRODUCTION

According to the competitive exclusion principle, two species cannot coexist when they occupy both the same ecological niche (Hardin, 1960). When this situation arises, even the smallest difference in competitive ability will result in the elimination of the less competitive species (Hardin, 1960). If two species appear to contradict this principle, it is likely that there is a difference in their life-history strategies that allows them to coexist (Hardin, 1960).

The bullhorn acacia tree, *Acacia collinsii*, is part of a well-documented mutualism with several species of ants including *Pseudomyrmex spinicola* and *Crematogaster brevispinosa*. The tree supplies food for the ants in the form of beltian bodies and extrafloral nectaries and shelter in the form of hollow thorns called domatia. In turn, the ants defend the acacia tree from herbivores and trim encroaching vegetation. Of these ant species, *P. spinicola* individuals are larger and more aggressive (Aho et al., 2011), and one would therefore expect *C. brevispinosa* to be excluded from areas inhabited by *P. spinicola*. Despite an apparent competitive disadvantage, *C. brevispinosa* colonies persist in areas populated by *P. spinicola*, which

locally controls approximately 70% of acacias (Wilson et al., 2004).

To investigate the apparent contradiction of the competitive exclusion principle we compared two life-history characteristics between the Acacia ants: dispersal ability and reproductive output. We suspect that an advantage in either would permit *C. brevispinosa* to coexist with *P. spinicola*. The ability to colonize younger trees could allow *C. brevispinosa* to utilize resources before being competitively excluded by *P. spinicola*, and the ability to produce more larvae per unit volume could permit *C. brevispinosa* to outnumber and thus outcompete *P. spinicola* in territorial battles.

METHODS

We selected five 15m x 15m plots in stands of *Acacia collinsii* containing both *Crematogaster brevispinosa* and *Pseudomyrmex spinicola* in Palo Verde National Park, Guanacaste, Costa Rica, on 09 Jan, 2012. We arbitrarily classified *A. collinsii* trees above and below 1 m as mature and young, respectively. We surveyed every *A. collinsii* stem in each plot, noting the age class of the tree and which ant species was present. We

defined trees living without mutualistic ants as uninhabited.

To quantify reproductive output, we collected a total of four domatia for each species of colonizer ant. We initially collected 4 domatia from *P. spinicola* colonies and 2 domatia from *C. brevispinosa* colonies at our study site. To equalize our sample, we collected 2 additional domatia from *C. brevispinosa* colonies at a secondary site 5 kilometers away. From mature *A. collinsii* trees, we selected domatia that showed high ant activity (excluding those that did not contain larvae). We calculated domatia volume (V) by estimating the shape of a domatium as a pair of perfect cones, using measurements of thorn length (l_1 and l_2) and diameter at the widest point (d):

$$V = \frac{1}{3}\pi\left(\frac{d}{2}\right)^2(l_1 + l_2)$$

We quantified larval density by estimating the mean number of larvae per unit volume of domatia.

Data Analysis

We compared the distribution of the two ant species we observed in young trees to the expected distribution measured in mature trees using a chi-squared test. We used a t-test to compare larval density between species. The larval density data were log-transformed for normality. Reported means and confidence intervals were back transformed. All statistical analyses were performed using JMP 9 software (SAS Institute Inc. 2010).

RESULTS

We found that neither species was significantly more likely to be present in young trees relative to its proportion in mature trees ($\chi^2 = 0.281$, $df = 1$, $P = 0.60$; Fig. 1). Additionally, young trees were less likely to contain ant colonies than mature trees ($\chi^2 =$

6.852, $df = 2$, $P = 0.03$). Lastly, larval density was higher in domatia of *C. brevispinosa* colonies than in those of *P. spinicola* ($t = -2.77$, $df = 3.62$, $P = 0.028$; Table 1; Fig. 2).

DISCUSSION

Neither *C. brevispinosa* nor *P. spinicola* inhabited more young *A. collinsii* than expected based on their frequency in adult trees. Thus, differences in colonization ability do not explain their coexistence. Contrary to Janzen's (1983) proposal that *P. spinicola* abandon aging trees in favor of young trees with more valuable resources, our data showed that mature trees rather than young trees were more likely to be inhabited by ants of both species. This refutes our hypothesis that *C. brevispinosa* persists by dispersing to young trees.

We found that *C. brevispinosa* produced more larvae per unit volume than *P. spinicola*. This reproductive advantage may allow *C. brevispinosa* to produce larger colonies than *P. spinicola*. In a study of competitive acacia ants in Africa, Palmer (2003) determined larger colonies generally succeed in territorial battles, even against more aggressive species. This suggests that a large *C. brevispinosa* colony could outcompete a small *P. spinicola* colony despite differences in aggression between species.

Although we found a significantly higher larval density in *C. brevispinosa* domatia, we did not examine the distribution of larvae among domatia within individual trees. *C. brevispinosa* may concentrate larvae among a few domatia, while *P. spinicola* may distribute them more evenly within its host trees. If distribution of larvae among domatia differed, we could have overestimated the difference in reproductive output between the two ant species. Furthermore, we studied the reproduction of these ants on a very short temporal scale. Differences in reproductive cycles

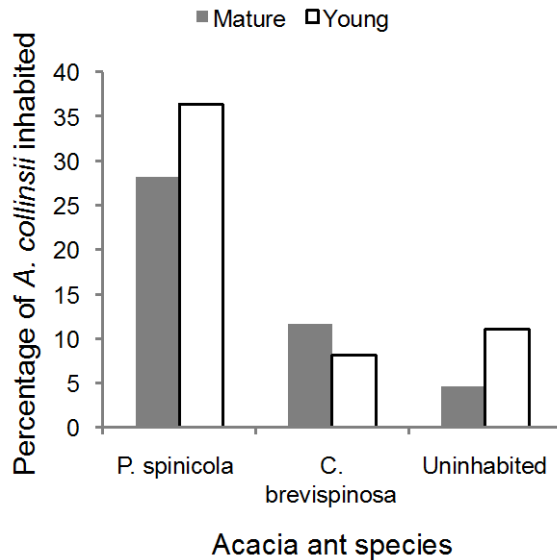


FIG 1. Occupation of mature and young *A. collinsii* by *P. spinicola* and *C. brevispinosa*. Young trees were more likely to be uninhabited than were mature trees, but species distributions did not differ as a function of tree age. This figure is for illustration purposes only and does not reflect our statistical analysis of the data which was based on chi-square.

might allow for a dynamic equilibrium in which ants achieve peak reproductive rates at different times. Despite these limitations, the differences in larval density between ant species are notable and offer a compelling argument for the persistence of *C. brevispinosa* alongside *P. spinicola*.

TABLE 1. Mean larval density for two acacia ant species

Species	Mean larval density (larvae/cm ³)	Upper 95% CI	Lower 95% CI
<i>Crematogaster brevispinosa</i>	190.5	3042.3	12.5
<i>Pseudomyrmex spinicola</i>	15.7	38.3	6.4

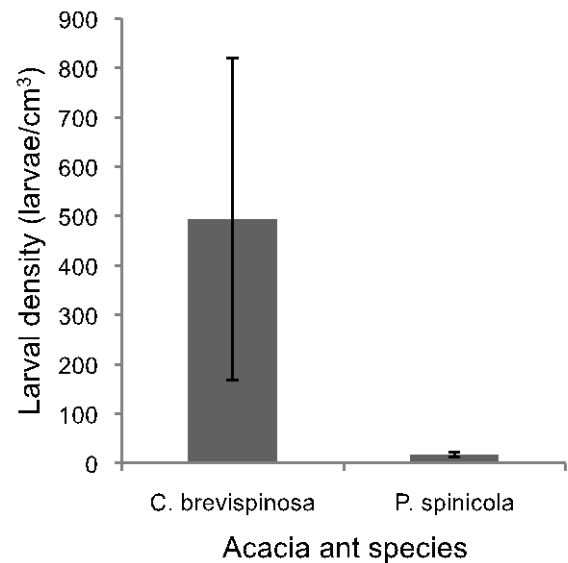


FIG 2. Larval density of two ant species in *A. collinsii* domatia showing higher larval density in *C. brevispinosa* colonies than in *P. spinicola* colonies. Error bars show standard error.

We conclude that the coexistence of *C. brevispinosa* and the *P. spinicola* is not a contradiction of the competitive exclusion principle. Rather, we suspect that the higher reproductive output of *C. brevispinosa* enables it to compete with its more aggressive rival *P. spinicola* for control of acacia trees.

This difference in the life strategy of *C. brevispinosa* may explain why it can occupy the same niche as *P. spinicola*, and still maintain a viable population.

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VARYING INVESTMENT IN DEFENSE WITH *ACACIA COLLINSII* AGE

ANNA L. DEFFEBACH, SHEA E. FLANAGAN, NINA B. FRANKEL, AND MILO S. JOHNSON

PROJECT DESIGN: RYAN CALSBEEK. FACULTY EDITOR: RYAN CALSBEEK

Abstract: Plants allocate investment in defense over their lifetime to maximize individual fitness. *Acacia collinsii*, a swollen-thorn acacia tree, forms an obligate mutualism with the ant *Pseudomyrmex spinicola* that in return for food and shelter, protects the tree. Here we examine the dynamics of *A. collinsii* investment in defense over time, both in regard to tree resource allocation and ant colony health. We predicted that younger trees would provide higher densities of ant resources and would house healthier ant colonies, as younger trees may need a greater level of defense from herbivory and interspecific competition. However, we found that older trees had a higher density of ant shelters (domatia). Older trees also had larger ants, independent of the abundance of domatia, suggesting that older trees may invest more in ant provisioning to maximize reproductive fitness. Our results indicate a dynamic mutualism in which defense investment changes over time, a potentially complex system that merits further study.

Key Words: resource allocation, myrmecophyte, *Acacia collinsii*, *Pseudomyrmex spinicola*, domatia, herbivory

INTRODUCTION

According to the optimal defense hypothesis, organisms allocate investment in defense over their lifetime to maximize individual fitness, by both surviving to reproductive age and remaining healthy enough to reproduce (Stamp 2003). Stamp (2003) described a strategy in which organisms invest in defense in direct proportion to the risk of high cost herbivory. Younger plants are at a higher risk from herbivory due to the plants' small stature and relatively low amount of expendable plant biomass. However, another possible optimal defense strategy is to increase defense at the reproductive stage to ensure maximum fecundity, resulting in increased investment in defense among older plants.

Instead of relying on chemical or mechanical plant defenses, the tree *Acacia collinsii* has evolved an obligate mutualism with the ant *Pseudomyrmex spinicola* for defense. In this mutualism, the ants protect the tree from herbivory and interspecific competition while the acacia tree provides the colony with large stipular thorns (domatia) that serve as shelter for ants and larvae, and modified leaflet tips

(Beltian bodies) and foliar nectaries to provide food (Janzen 1966). While the defensive ants replace chemical and mechanical defenses in this mutualism, the optimal defense hypothesis still applies (Holland et al 2009).

We investigated how *A. collinsii*'s investment in ant defense varied with tree age. We used the density of domatia that *A. collinsii* provided for *P. spinicola* as an indicator of the plant's investment in defense. We predicted that *A. collinsii* would invest more in resources for the ants at a younger age, because a young acacia without ant protection is highly vulnerable to costly insect and rodent attacks (Janzen 1966). We further predicted that increased investment in ant resources at a younger age would translate directly to a more successful ant colony with greater ant size (length).

METHODS

We haphazardly sampled 28 trees inhabited by *P. spinicola* in an early successional field in a dry forest on the morning of 9 January 2012, in Palo Verde National Park, Guanacaste Province, Costa Rica.

Tree diameter at breast height (DBH) is a proxy for tree age (Condit et al. 1993). We measured tree diameter with calipers to the nearest 0.1 mm. We measured domatia densities by counting all domatia occurring within the 20 cm vertical 180° plane at breast height. For each tree, we captured 5 ants using duct tape and measured each ant's body length to the nearest 0.1 mm with calipers.

Tree DBH and domatia density were log-transformed to improve normality of their distributions. We used a multiple regression to separate the effects of DBH and domatia density on mean ant size. All statistical analyses were performed using JMP 9.0 (SAS Institute, INC, Cary, NC 2011).

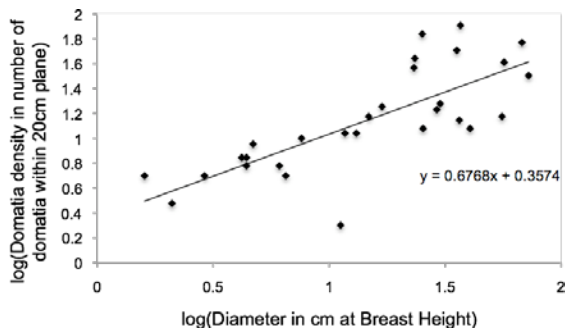


Fig. 1. *A. collinsii* size (DBH) was positively correlated with domatia density ($r^2_{27}=0.59$, $P=0.0001$).

RESULTS

Log-transformed DBH and log-transformed domatia density were positively and significantly correlated ($r^2_{27}=0.59$, $P=0.0001$; Fig. 1). Ant size was significantly correlated with log-transformed DBH after holding the effects of log-transformed domatia density constant using a multiple regression (ANOVA $F_{1,25}=14.68$, $P=0.0008$; Table 1). Log transformed domatia density was not a significant effect in this model (ANOVA $F_{1,25}$, $P=0.30$).

DISCUSSION

We examined whether *A. collinsii* tree age (i.e., DBH) had an effect on resource availability for their ant mutualists. Our results showed that domatia density increased in older trees, which contradicted our original hypothesis. We also found that older trees (i.e., larger DBH) housed larger ants and that after controlling for the effect of DBH, domatia density was not a good predictor of ant size.

It appears that *A. collinsii* follow the optimal defense strategy of maximizing defense during the reproductive stage to increase fitness, in contrast to our prediction of investing most in defense when young. Older trees also housed larger ants, but further studies are needed to determine if larger ants indicate a better defense force for the tree.

Although we found that older trees had a higher domatia density and older trees housed bigger ants, our data suggest that domatia density is not a good predictor of ant size. Even when older trees invest more in ant resources to increase defense, certain ant resources may more directly increase ant size than others. Beltian bodies provide protein for the ants, and thus may predict ant size (Janzen 1983). We were not able to measure the density of Beltian bodies in this study because they are not present in the dry season, when we conducted our experiment.

Plants that utilize mutualistic ant defenses exhibit differential investment in defense throughout their lifespan, as do plants with chemical or mechanical defenses against herbivory. Changes in investment reflect which stage of a plant's life requires the most protection. Future studies are needed to further illuminate the dynamics of plant optimal defense theory in ant-plant mutualisms.

Table 1. *Acacia collinsii* size (DBH) significantly predicted mean ant size when controlling for the effect of domatia density. Domatia density was not a significant effect in this model.

Source	Estimate	Degrees of Freedom	Sum of Squares	F-Ratio	P-Value
log(Diameter at Breast Height)	0.81±0.21	1	1.70	14.68	0.0008
log(Domatia Density)	-0.25±0.24	1	0.13	1.10	0.3

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THE LIMITATIONS OF DIVERSITY INDICES: EDGE EFFECTS AND BUTTERFLY DIVERSITY

ROBIN A. COSTELLO, ANNA L. DEFFEBACH, MADELINE K. KREHER, JAMIE E. MCCLAUGHLIN

Project Design: Nina Lany. Faculty Editor: Ryan Calsbeek

Abstract: Given the increasing human-induced fragmentation of natural habitats around the globe, understanding the effect of fragments and their edges on biodiversity is important for developing effective conservation strategies. Edge habitats typically show greater variation in light levels, temperature, humidity, and wind. Experiments have attempted to quantify the effect of edge habitats on biodiversity, but results have been mixed. To measure the effects of edge habitat on biodiversity, we observed the number of butterfly species in edge and interior forest plots at Palo Verde National Park, Costa Rica. We chose butterfly diversity as a proxy for total biodiversity because butterflies are a well-established indicator species. Because edge habitats are defined by variable abiotic factors, we expected edge habitats to provide more niches and therefore greater biodiversity. We hypothesized that butterfly species richness and evenness would be greater in edge plots than in plots in the interior of the forest. We found greater species richness in edge habitats, but greater species evenness in interior plots. These findings emphasize the importance of including a variety of habitat types when conserving biodiversity.

Keywords: Edge effect, biodiversity, species richness, butterflies, Simpson biodiversity index

INTRODUCTION

As human influence expands to more remote areas, maintaining global biodiversity becomes increasingly important. This expansion of human influence rarely damages natural habitats uniformly; more often habitats are broken up into more or less pristine fragments that are separated by developed land (Noss 2006). This habitat fragmentation reduces both the area covered by the habitat type and changes the habitat configuration (Harris 1984). Due to these effects, human induced habitat fragmentation has been shown to reduce biodiversity (Noss 2006). This increase in habitat fragmentation by definition leads to an increase in the amount of edge habitat. Edge habitat is different from the interior habitat of the fragment for a range of reasons including greater fluctuation in levels of light, temperature, humidity and wind (Kapos 1989). Interestingly, the role

of these “edge effects” on biodiversity is not well understood by the conservation biology community. Many scientists believe that a change in habitat from a stable environment to an edge characterized by variability leads to a loss in biodiversity (Primack 1995). However, experiments that have attempted to quantify edge effects on biodiversity have found a range of results, from decreasing to increasing biodiversity relative to the interior (Pin Koh 2007). A possible reason for this lack of clarity is a lack of effective methodology.

Comprehensively monitoring tropical biodiversity requires a large amount of time and funding, leading conservation biologists to search for less costly methods. Using indicator species as a proxy for overall biodiversity is a relatively easy, inexpensive and effective method of estimating biodiversity (Bonebrake et al 2010). While no species is a perfect indicator for overall habitat

quality, butterflies have commonly been used as an indicator due to their sensitivity to environmental change, short generation time, and well-understood evolutionary history (Bonebrake et al 2010; Pin Koh, 2007).

We measured butterfly diversity in edge and interior plots in the dry tropical forest of Palo Verde National Park, Costa Rica to quantify differences in biodiversity. Metrics of biodiversity involve a measure of both species richness and evenness (Magurran 2004). Because edge habitats are characterized by variability, we predicted that edge habitats would provide more niches. Therefore, we expected our edge plots to have a greater biodiversity, supporting both a greater species richness and evenness.

METHODS

We established four study plots in the tropical dry forest of Palo Verde, Costa Rica (10.352°N, 85.308°W) on January 10 and 11, 2012. We selected two plots of edge habitat and two plots in the interior of the forest, keeping elevation constant. Edge plots were located within 10 meters of a dirt road bordering a cattle pasture habitat. To quantify the transition from forest to pasture, we measured vegetation density and canopy cover in the edge and interior plots. We estimated these metrics in all four cardinal directions at three different points at each plot (one at the middle of each transect and one in the middle of the 100m buffer between transects). To measure vegetation density, we tied a cloth bandana 2 m up a 3 m pole and walked with the pole until a stationary observer standing in the

middle of the transect could no longer see the bandana. We measured canopy cover using a concave spherical densiometer. To test for a significant difference in vegetation cover between plots, we performed a two-way *t*-test using JMP 9 (Cary, NC).

To assess butterfly diversity, we walked two 100m transects in each plot. The two transects within each plot were separated by 100m. We assigned individual butterflies along the transect into distinct species, by visually noting color patterning and shape to differentiate among species. Individuals were hand-netted for closer inspection when necessary. Although we differentiated among butterfly species, we did not taxonomically identify all butterfly species. We catalogued the number of visually distinct butterfly species in edge versus interior plots and the overlap of species between the two habitats. To test whether our sampling was robust enough to account for total butterfly diversity, we plotted a species accumulation curve for each habitat. To graphically compare butterfly diversity between edge and interior plots, we generated rank abundance curves for our study areas. To quantify biodiversity between the habitats, we used the Inverse Simpson Index, which is calculated using the following equation:

$$1/D = (1/\sum \frac{n_i(n_i-1)}{N(n-1)})$$

Where *D* is diversity, *n_i* is the number of individuals in the *i*th species and *N* is the total number of individuals (Magurran 2004).

Species evenness was then calculated using the equation:

$$E = \frac{(1/D)}{S}$$

Where D is equal to diversity and S is equal to the number of species in the sample. To assess the difference between the Inverse Simpson Index measures, we conducted a sensitivity analysis. In this analysis, we manipulated species abundances to find the number of species that would need to be removed from the edge plots to make the diversity index equal between the two habitats.

RESULTS

Canopy cover was significantly higher in the interior plot than in the edge plot ($t_5=-4.63$, $P < 0.01$), but there was no significant difference in vegetation density between the two plots ($t_5=0.79$, $P=0.23$). We counted 40 species of butterfly, 17 of which were present in all four plots, 17 present only in the edge plots, and 6 present only in the interior plots (Fig 1). We observed a total of 34 species in the edge plots and 23 in interior plots. A species accumulation curve indicated that few new species were found in interior plots by our final visit. New species were still being found in edge habitat by our final visit. However, this number was beginning to plateau (Fig 2). Rank abundance curves demonstrated that all plots were dominated by a few species, although the edge plots contained both more individual butterflies and more species (Fig 3). Sensitivity analysis revealed that we would need to have found 9 more species in the interior

habitat to equalize the Inverse Simpson Biodiversity Index between edge and interior plots. The inverse Simpson's index was 7.74 for interior habitat and 8.83 for edge habitat. Evenness was 0.336 for interior habitat and 0.260 for edge habitat.



Fig 1. Number of butterfly species distributed among edge and interior forest plots. There were more total butterfly species in edge habitat than in the interior.

DISCUSSION

We found greater butterfly diversity in our edge plots than in our interior plots. Although the Inverse Simpson's Indices appeared similar, we would have

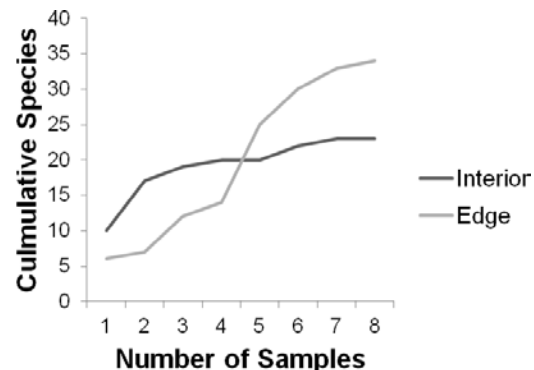


Fig 2. Species accumulation curves for each habitat type showing that few new species were found by our final visit to interior plots, and though new species were still found in edge plots, this number began to plateau.

had to find 9 more species in the interior plots before interior forest diversity matched edge diversity.

These data support our hypothesis that species richness is greater in edge plots than in interior plots. However, interior plots exhibited higher species evenness compared to edge plots.

Higher species richness in edge habitat could be explained by the variability of edge habitats. The reduced canopy cover found in the edge plots, for example, may have generated greater spatial variation in temperature and light. This variation may provide more niches, which is predicted to lead to greater species richness (Groom et al 2006).

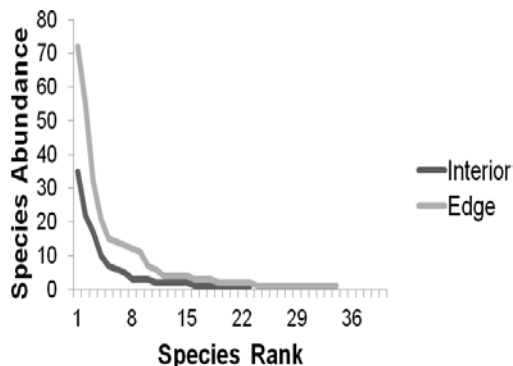


Fig 3. Rank abundance curves for each habitat type illustrating that both edge and interior habitats were dominated by a few butterfly species, but edge habitat contained both more species and more individual butterflies.

Interior plots, where abiotic factors such as light, humidity, and temperature are more uniform, exhibited greater evenness than the edge plots. This stability in the environment enables a few species to distribute themselves equally (Bonebrake et al 2010). Our results seem to indicate that biodiversity increases with habitat fragmentation. However, our results also reveal that different habitats support different components of

biodiversity. Because ecologists still know little about how evenness and richness interact to influence ecosystem stability, attributing a high biodiversity to a single habitat is a naïve conservation goal (Groom et al 2006).

ACKNOWLEDGEMENTS

We would like to thank Nina Lany for qualitatively describing the plant composition of our four study plots.

AUTHOR CONTRIBUTIONS

All authors contributed equally.

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HEAD-BOBBING AND SEXUAL SELECTION: THE EFFECTS OF SIZE ON COURTSHIP AND TERRITORIALITY IN *CTENOSAURA SIMILIS*

HEMAYAT R. CHOWDHURY, SHEA E. FLANAGAN, NINA B. FRANKEL, MILO S. JOHNSON, AND
BENJAMIN J. KESSLER

PROJECT DESIGN: MICHAEL L. LOGAN. FACULTY EDITOR: RYAN CALSBEEK

Abstract: Due to the unequal reproductive costs for males and females in most vertebrates, females must be more discerning than males in choosing mates. As such, males have evolved several strategies for attracting females and competing with other males for mates. Head-bobbing, a frequently observed behavior in the iguanid *Ctenosaura similis*, is thought to be one such mechanism, though its exact purpose is not well understood. To better understand how this behavior relates to sexual selection and territoriality, we observed the relationships between head-bobbing in male *C. similis* as a function of age class and the number of male or female lizards in the vicinity of the focal male. Additionally, we examined whether territory size varied as a function of male body size. We found that larger male *C. similis* patrolled larger territories and head-bobbed more frequently compared to smaller males. Head-bobbing did not relate to the number of males or females in the vicinity of the focal male. We conclude that larger males use head-bobbing to demonstrate their dominant status, and that this status represents their ability to defend a larger territory or gain more mating opportunities.

Key Words: sexual selection, territoriality, *Ctenosaura similis*, head-bobbing

INTRODUCTION

On average, the cost of reproduction is thought to be greater for females than males, because female gametes are both larger and fewer in number than male gametes (Alcock 2005). Additionally, egg gestation occurs within the females of most species (some of which must also provide parental care), which further increases the costs associated with reproduction (Alcock 2005). Sexual selection theory predicts that these female-specific reproductive costs should lead to “choosy” females and male-male competition for access to mates. A choosy female is one that best ensures the survival and future reproductive success of her offspring by mating with only high-quality males. Thus, females often choose mates based on traits that demonstrate high male fitness, such as size, territory quality, social dominance or physical strength (Ryan 1994). In many species, male courtship displays have evolved as a way to demon-

strate these attributes to females. Moreover, since the pool of available females is often limited, males may compete with one another for mating opportunities (Alcock 2005).

The large iguanid *Ctenosaura similis* is sexually dimorphic; males are larger than females in body size and possess more pronounced dorsal spines and dewlaps (a flap of skin hanging below the chin and throat). These secondary sexual characteristics are even more pronounced on larger males than smaller males. Both male and female *C. similis* exhibit head-bobbing behavior, although males are thought to exhibit this behavior with greater frequency. Head-bobbing is believed to be involved in territoriality and courtship (Savage 2002).

We examined how the function of male *C. similis* head-bobbing behavior varies as a function of age class. We hypothesized that older male *C. similis* would display more head-bobbing behavior and have a larger territory than younger males. We also hypothe-

sized that males would head-bob more often in the presence of other males as a display of territoriality (male-male competition) and in the presence of females as a courtship display (female choice). To test these hypotheses, we observed head-bobbing behavior in a population of adult and sub-adult male *C. similis* at the Palo Verde Biological Station, Costa Rica.

METHODS

On 11 January 2012 from 0800-1130 at the Palo Verde Biological Station, Costa Rica, we located ten male *C. similis* near the premises, and classified them as adult or sub-adult. We considered “adult” males to be individuals with a snout-vent length (SVL) of over 400 mm (and bearing prominent dorsal spines), and “sub-adult” males to be individuals with an SVL of less than 400 mm but still bearing small dorsal spines. We observed each lizard for five ten-minute focal periods separated by two-minute breaks. We remained 10-15 m away from the focal individual, and began observations ten minutes after arrival to ensure that the lizard had acclimated to our presence.

We recorded the furthest distance between two points traveled by the focal lizard over the observation period as an estimate of territory size. We also estimated SVL by comparing the lizard’s snout and vent to nearby reference points on the ground or in photographs taken by the observer, and measuring length between the reference points with a ruler. At the beginning of each ten-minute focal period, we counted the numbers of male and female lizards present within a 10 m range of the focal animal. During each focal period, we counted the total number of head-bob clusters. We defined a “cluster” as a distinct period of head-bobbing that was separated from other clusters by at least 10 se-

conds. Additionally, we noted any behaviors other than head-bobbing that we observed.

Statistical Analyses

We used linear regression to test for relationships between 1) territory size and SVL, 2) SVL and total number of head-bob clusters, 3) mean number of females within 10 m and mean number of head-bob clusters, and 4) mean number of males within 10 m and mean number of head-bob clusters. For regressions involving the number of females and males within 10 m, we averaged the number of females present, males present, and head-bob clusters, over the entire one-hour observation period for each lizard. Additionally, we used a two-tailed t-test to compare the mean number of head-bob clusters between age classes. In cases where results were not significant, we performed power analyses to determine whether our small sample size prevented us from seeing a biologically significant pattern. All analyses were conducted using JMP 9 (SAS institute, Cary NC).

RESULTS

Snout-vent length and distance traveled were positively correlated ($r^2=0.66$, $P=0.0178$; Fig. 1). Adult males ($n=4$) performed a greater number of head-bob clusters than sub-adult males ($n=6$) ($t_{7,2}=4.19$, $P=0.0019$, Fig. 2), and males with larger SVLs tended to have more total head-bob clusters, though this result was not significant ($r^2=0.31$, $P=0.097$). There was no significant correlation between the mean number of head-bob clusters and mean number of males ($r^2=0.002$, $P=0.89$) or females ($r^2=0.029$, $P=0.64$) within 10m of the focal *C. similis*. Lastly, there was no significant correlation between SVL and the mean number of males ($r^2=0.068$, $P=0.47$) or females ($r^2=0.16$, $P=0.26$) within 10 m of the focal *C. similis*.

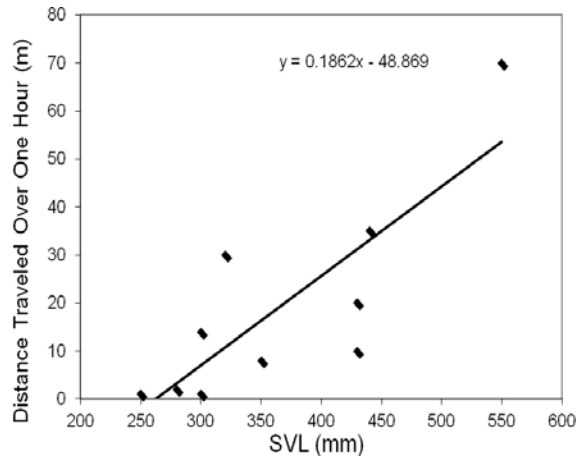


Fig. 1. The maximum distance traveled between two points during the observation period (a proxy for territory size) as a function of SVL.

DISCUSSION

Adult males head-bobbed more often than sub-adult males, and there was a trend towards more total head-bob clusters with increasing body size on a continuous scale. These results indicate that head-bobbing behavior could be an indicator of elevated social status in the older males. Moreover, larger males traveled longer distances during our observations, suggesting that they patrol larger territories and protect more resources. Alternatively, larger males may be more sexually active, spending more time roaming and searching for potential mates.

Unfortunately, we lacked sufficient sample size to detect the effects of nearby conspecifics on head-bobbing. Our tests of the effects of nearby males or females on head-bob clusters lacked sufficient power to detect relationships between these variables (power = 0.0519, power = 0.0718, respectively). Nevertheless, head-bobbing frequency may not have been related to the presence of conspecifics, and in that case a larger sample size would have still failed to reveal a relation-

ship. Although our data do not allow us to draw meaningful biological conclusions regarding head-bobbing in the presence of conspecifics, our personal observations and previous studies suggest that head-bobbing does occur in a social context (Janzen 1983, Savage 2002). Interestingly, Janzen (1983) noted that *C. similis* head-bobs in a species-specific pattern, but we observed individual-specific head-bobbing patterns in our *C. similis*. A future study could investigate variation in individual head-bobbing patterns and their potential relationship with individual reproductive success. Further work should include more observations of *C. similis* individuals to better distinguish between potential alternative functions of head-bobbing behavior such as territory defense and courtship.

Sexual selection produces many mysterious behaviors that at first seem illogical, as they do not appear to help ensure an individual's survival. Instead, it is likely that traits such as head-bobbing persist because they increase the reproductive success of the individuals bearing them. The fruits of sexual selection, driven by female choice and male-male competition, provide us all with beautiful examples of diversity and countless opportunities for scientific inquiry.

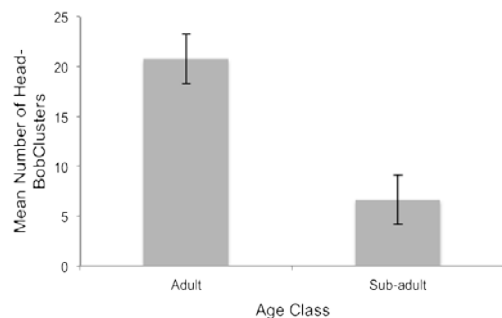


Fig. 2. Mean head-bob clusters for adult and sub-adult *C. similis*.

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EFFECTS OF RESOURCE AVAILABILITY ON THE DISTRIBUTION OF *ASTYANAX AENEUS*

WALES A. CARTER, MADILYN M. GAMBLE, JESSE T. RIEB, AND AMY E. VAN SCOYOC

Project Design: Ryan G. Calsbeek. Faculty Editor: Ryan G. Calsbeek

Abstract: The ideal free distribution (IFD), when applied to biological systems, states that organisms will distribute themselves in such a way that each individual maximizes its own energy intake. In this study, we exposed *Astyanax aeneus*, an omnivorous freshwater characid fish, to a series of treatments in which food resources were unevenly distributed. We hypothesized that we would observe feeding behavior consistent with IFD. Our results were not consistent with the hypothesis that *A. aeneus* would distribute themselves according to the IFD. Instead, our data suggest that *A. aeneus* shows territorial behavior that precludes the IFD.

Key words: *Astyanax aeneus*, ideal free distribution, territoriality, Guanacaste Province Costa Rica

INTRODUCTION

The ideal free distribution (IFD) predicts that individuals in a population will distribute themselves across all available resource patches, regardless of quality, in such a way that each maximizes its own access to resources (Fretwell and Lucas 1970). The IFD assumes that as the size of a resident population increases, the suitability of a given patch decreases, and that there will be an equilibrium density at each patch so that the net rate of food uptake for a given individual will be equal regardless of which patch it inhabits. Finally, it requires that the individuals are able to recognize the best patches and that they are free to move to the best available patch. Although there is some debate about its applicability to natural systems, the IFD hypothesis nevertheless provides a useful framework from which we can evaluate the characteristics of animals that lead to such specific behaviors (Krivan et al. 2007).

Fish provide a useful system with which to test the IFD and its assumptions. Fish live in a more or less uniform medium, which allows free movement and may facilitate the recognition of patches of varied quality. Due to its generalist feeding behaviors (Bussing

1998), we hypothesized that *A. aeneus*, a widespread omnivorous freshwater characid fish, would exhibit behaviors characteristic of the IFD by changing its distribution in response to changes in food distribution.

METHODS

On 10 January 2012 we used a seine net to trap approximately 30 *A. aeneus* from a watering hole in Palo Verde National Park, Guanacaste Province, Costa Rica (10.392°N, 85.308°W). We acclimatized the fish to well water over a 24 hour period during which we withheld food to ensure their responsiveness to our food treatments. We conducted our experiment in a 35 x 60 cm black storage container divided by a line of marking tape into two halves (sides "A" and "B") and filled with approximately 15 cm of water. We selected four fish to provide a range of sizes, from small to large (coded as 1-4, respectively), and introduced them to the center of our test tank. We then performed four 5-minute feeding treatments with the selected fish, each followed by a 5-minute rest period to allow food to settle and fish to return to a non-feeding distribution. Our food-treatment con-

sisted of a suspension of powdered cornflakes in water and was delivered by pipette.

The first treatment consisted of a control in which no food was added to either side of the tank. In the second treatment we added 3 drops of food to side A and 1 drop of food to side B every 30 seconds, and in the third treatment we added 1 drop of food to side A and 3 drops of food to side B every 30 seconds. The final treatment was a second control in which 2 drops of food were added to both side A and side B every 30 seconds. At 20 second intervals during these treatments, we recorded the total number of fish on side A and the size codes of those fish. We replicated this series of treatments twice and then replicated both of the experimental treatments a third time. New fish were used in each replicate.

Statistical Analysis

We conducted an ANOVA to compare the mean number of fish on side A for each treatment. We used a one sample two-tailed t-test to test for systematic unevenness in mean fish distribution between side A and side B across treatments. Next, to investigate an observed trend of territoriality (below) we used an Ordered Heterogeneity Test (Rice and Gaines 1994) to determine how fish size class influenced the percent time greater than 50% spent on the preferred side of the tank. We defined the preferred side for each fish as the side on which that fish spent more time on average during the entire series of treatments. All tests were conducted using the JMP 9 statistical package (SAS Institute, Cary, NC).

RESULTS

We found no significant difference in distribution of fish between any of the treatments ($F_{3,9} = 0.42$, $P = 0.74$). There was a significantly greater number of fish on side A during both the feeding and control treatments (mean = 2.58, $t_9 = 3.42$, $P = 0.008$; figure 1).

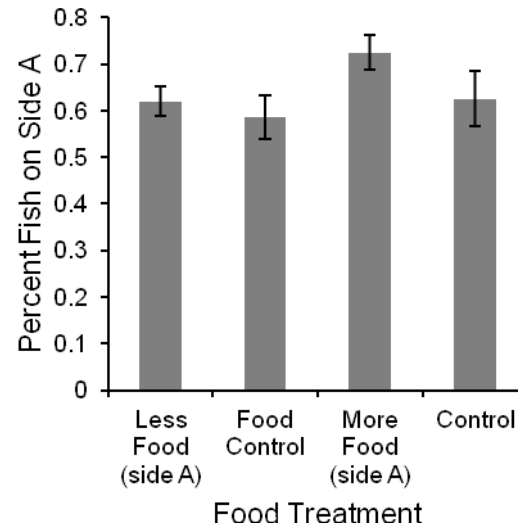


Fig 1. There were no significant differences in *Astyanax aeneus* distribution among treatments. The error bars represent standard error.

Individual fish did not spend equal amounts of time in each side of the tank. On average, individuals were found on their preferred side in $72 \pm 8\%$ of sample points (95% CI). Percent time greater than the expected 50% on the preferred side increased with relative fish size, though this trend was not statistically significant (Ordered Heterogeneity Test: $r_s P_c = -0.36$, $P = 0.12$; figure 2).

DISCUSSION

We found no indication that fish exhibit an IFD. Had the fish exhibited behavior in accordance with the IFD we would have expected the distribution of fish to mirror the

distribution of food resources in experimental treatments, and the distribution of fish in experimental treatments to be different from the distribution of fish in control treatments.

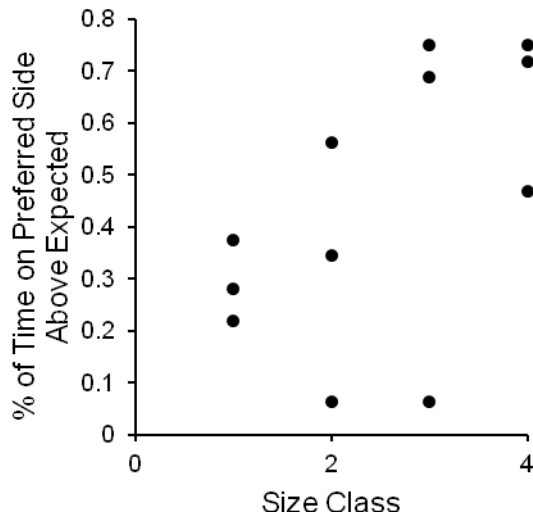


Fig 2. A statistically non-significant trend indicating that proportion of time spent on a fish's preferred side increases with fish size. Points indicate mean time spent on the preferred side across all four treatments.

However, there was no significant difference in the percent of *A. aeneus* on "side A" of the tank between any of the four treatments indicating that the treatments did not affect fish distribution. In fact, individual fish did not spend equal amounts of time on both sides of the tank. The mean proportion of time spent on side A was significantly greater than our expected 50%, across both experimental and control treatments. This result indicates that *A. aeneus* distribution is not uniform even within a uniform environment. We conclude that in situations where fish size is heterogeneous *A. aeneus* do not exhibit feeding behavior in accordance with the IFD.

We observed a non-significant trend suggesting that the largest fish spent a greater proportion of time on its preferred side com-

pared to smaller fish, regardless of treatment. This suggests that larger fish may establish a territory in one part of the tank and stay in that territory regardless of changes in resource availability, while smaller fish seem to distribute themselves more in line with the IFD with respect to food. Alternately, it is possible that in populations with fish of equal size, no one fish is able to establish a territory and so all fish distribute themselves optimally with respect to food. In both of these cases, the freedom of fish to move between resource patches is essential to allow fish to redistribute themselves at will. The fact that we found a lack of free movement in our experiment may be one of the best explanations for why we did not observe an IFD.

Although our data did not support an IFD, it is possible that measurement error may have been introduced by the subjective selection of fish size classes. Additionally, as we did not note the sex of the fish used, we may have inadvertently introduced mate availability as a confounding variable. Also, the time-scale on which we observed fish distribution may not have been sufficient for the system to reach equilibrium. Finally, the assumptions of the IFD in natural systems, especially omniscience regarding resource distribution, may not have been met in this experiment. Future studies may wish to explore whether a more exaggerated difference in food availability between sides causes a trend towards an IFD in *A. aeneus*. To determine the validity of our assumption of omniscience, we suggest further investigation into the specific physiology and feeding cues of *A. aeneus*.

Our findings raise further questions about territoriality in *A. aeneus*, especially given that the preferred side of the largest fish in each replicate did not necessarily correspond to

greater resource availability. If these preferred sides could be considered territories, it is uncertain why the largest fish might protect an area that did not include the best resources. The benefits of defending a sub-optimal "territory" are unclear, though further research into the costs of territoriality and long-term trends in resource availability may help to clarify the benefit of this behavior. Although we found no suggestion of an IFD in *A. aeneus*, many factors must be considered before it is completely ruled out as a model for the IFD.

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DENSITY DEPENDENCE OF ANT LION (*MYRMELEON CRUDELIS*) TRAP CAPTURE RATES DOES NOT EXPLAIN SPATIAL DISTRIBUTION OF TRAPS

Robin A. Costello, Shea E. Flanagan, Madeline K. Kreher, and Jamie E. McLaughlin

Faculty Editor: Ryan Calsbeek

Abstract: Intraspecific competition for food is often a dominant influence driving the spatial distributions of predatory organisms in nature. Competition favors predators that can isolate themselves from their competitors. Ant lions (*Myrmeleon crudelis*), predatory terrestrial Odonate larvae that form conical sand traps to capture ants, more frequently occur in high density trap clusters than in isolated traps. To test the hypothesis that clustering offers an advantage in capturing prey that outweighs the potential costs of intraspecific competition, we collected ant lions, experimentally manipulated their trap densities in the laboratory, and recorded subsequent rates of prey capture. We found a statistically non-significant trend indicating that traps in low-density areas have greater prey capture success, which suggests that high-density clusters persist due to other pressures, such as limitation of suitable substrate or predation.

Key Words: Myrmeleon, intraspecific competition, spatial distribution, predation, habitat selection

INTRODUCTION

Identifying the drivers of spatial distribution is an important aim in ecology. Intraspecific competition for prey often drives the spatial distribution of predatory species (Hobbs and Munday 2004). When prey are limiting, predatory species usually compete for hunting grounds in which they attempt to maximize prey capture. Intraspecific competition thus leads to a change in spatial distribution, causing individuals to move away from each other to maximize hunting success. Ant lions (*Myrmeleon crudelis*) build stationary traps to capture prey, and are an ideal study system with which to examine how intraspecific competition influences spatial distribution.

Female ant lions lay eggs individually in dry, loose sand (Janzen 1983). Once hatched, larvae disperse in search of suitable hunting grounds (Janzen 1983). Ant lion larvae prefer fine grain sand relative to more coarse material (Grace et al. 2001). Individual larvae construct conical traps upon finding a

suitable habitat, in which they hide and wait for prey. Ant lions feed primarily on ants and other small arthropods that fall into their traps (Janzen 1983). Common predators of ant lion larva include small reptiles (Simon 1989), birds (Hauber 1999), and group-foraging ants (Lucas 1986).

Due to intraspecific competition, an individual ant lion in a low-density trap area is expected to have higher success at capturing prey, but most ant lion traps occur in high-density clusters in nature. Therefore, it is necessary to examine whether isolated traps increase prey capture success. An ant that moves to avoid one trap may fall into another. Therefore, we hypothesized that building traps in a cluster would increase the trap success of an individual and overcome the costs of intraspecific competition. To test this hypothesis, we manipulated ant lion larval densities in an experimental arena, and examined the relationship between trap density and trap success.

METHODS

On 13 January 2012, in Palo Verde National Park, Costa Rica, we measured the diameter, size of larva, and distance to the nearest neighbor for each trap along a 20 m roadside transect. We used linear regression to test for a relationship between larval size (a measure of success at acquiring food) and distance to nearest neighbor.

We collected fine grain sand, similar to that found at our field site, from the road outside the field station by sifting the substrate to remove rocks. We filled 6 experimental arenas with sand to a depth of 2.5 cm, controlling for arena length with dividers, and placed 2 temporary dividers to form a 6 cm buffer in the center of each arena. To control for variation in pit size in the experiment, we collected 40 ant lion larvae of similar size from traps between 2.5 and 3.5 cm, as trap diameter and larvae size are directly correlated (Janzen 1983). For each experimental arena, we released 1 ant lion into one end of the arena (the low density treatment), and 5 ant lions into the other end of the arena (the high density treatment). The high density treatment contained 5 ant lions to mimic the natural density of traps we observed, which ranged from 5 to 7 traps per cluster. All larvae formed traps over the next 8 hours.

The following morning (14 January 2012), we measured the diameter and distance to the three nearest neighbors for each trap. We defined trap density for each trap as the average of the distance to the three nearest neighbors. We collected ants of similar size and of the same species from our field site using aspiration and placed 30 ants in the middle of each of the 6 arenas after removing the temporary dividers. We recorded the number of captures per trap for 5 minutes; a "capture" was defined as an ant completely dropping into a trap. The observed

probability of trap success was calculated as the actual number of captures divided by the total number of ants captured by any trap in the arena. We ran this procedure once for all 6 experimental arenas.

We calculated the expected probability of an individual trap capturing an ant if all traps have an equal probability of success, given the proportion of ants captured by all traps in its arena as:

$$\text{Expected Success} = \frac{\text{ants captured by a trap}}{\text{total of ants in arena}} \cdot \frac{1}{\text{total of traps in arena}}$$

We then used a linear regression to compare expected trap success with observed trap success. We defined relative trap success as the residual observed trap success from this regression. We examined the relative success of a trap as a function of density (the average distance to the three nearest neighbors) using linear regression. Lastly, we examined the relationship between ant lion size and mean distance to nearest neighbor in the field using linear regression. Statistical analyses were carried out using the software package JMP 9.0 (SAS Institute, Cary NC).

RESULTS

The mean distance between an ant lion trap and its nearest neighbor in the field was 0.465 m (range = 0.04m to 1.92m), with over half of the traps sampled within 0.25 m of their nearest neighbor (Fig. 1). There was no relationship between ant lion size and the average distance to its three nearest neighbors in the field ($r^2=0.03$, $df=38$, $P=0.28$, Fig. 2).

In the laboratory experiment, the relative success of a trap was negatively related to trap density. However, this relationship was not statistically significant ($r^2=0.09$, $df=32$,

$P=0.09$, Fig. 3). All traps with a nearest neighbor distance greater than 10 cm captured more ants than expected, while clustered traps performed less well on average.

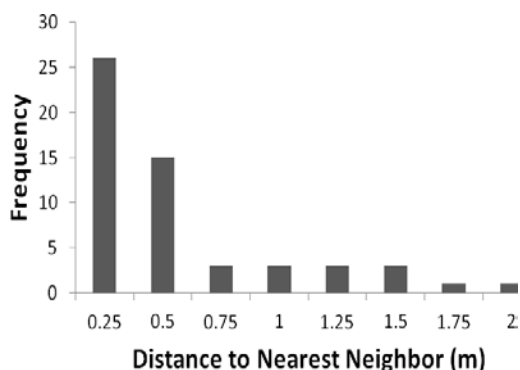


Fig. 1. Frequency of distance to nearest neighbor of 55 ant lion traps. The majority of ant lion traps in the field were clustered within 0.5 m of their nearest neighbor.

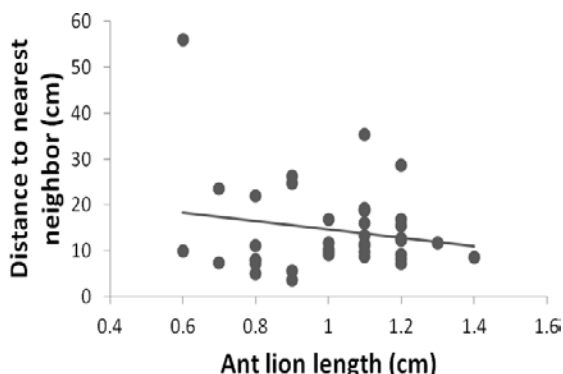


Fig. 2. Relationship between ant lion length and density. There was no significant relationship between larval ant lion length and the distance to nearest neighbor in the field.

DISCUSSION

We found a weak relationship between trap density and the probability of an ant falling into a trap. While not statistically significant, our data suggest that the success of traps built by larval ant lions increases with isolation. This indicates that ant lions with fewer neighbors experience less intraspecific

competition and can increase prey capture rates (Wilson 1984).

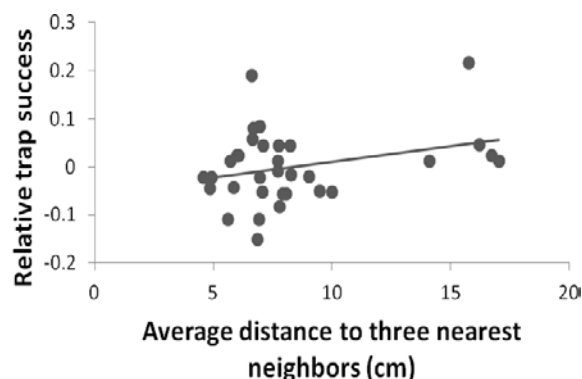


Fig. 3. Relationship between trap density and trap success. Relative trap success trended upward as average distance between the trap and its three nearest neighbors increased.

Despite the fact that ant lions capture more prey when isolated, the majority of traps in the field are located in high-density clusters. We did not find evidence that a limitation in dispersal ability could cause this clustering, as there was no correlation between size and trap density. Alternatively, ant lions may be clustered where appropriate substrate or prey items are abundant. Since ant lions prefer a fine soil substrate in which to make their traps (Berg et al. 1998), it is possible that ant lions are clustered in areas of fine soil, a limited resource in the rocky environment of Palo Verde National Park. Another potential explanation for the high frequency of clusters found in nature is a response to predation if high-density clusters lower the probability of being preyed upon for individuals within the cluster. Future work should distinguish between abiotic (substrate) and biotic (species interactions and predation) effects of ant lion distribution. Despite the significant pressures it poses, intraspecific competition cannot always fully explain the observed spatial distribution in nature.

AUTHOR CONTRIBUTIONS

All authors contributed equally.

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FACTORS INFLUENCING HABITAT SELECTION IN *NASUTITERMES CORNIGER*

WALES A. CARTER, M. HEMAYAT R. CHOWDHURY, NINA B. FRANKEL, AND AMY E. VAN SCOYOC

Faculty Editor: Ryan Calsbeek

Abstract: Investment in building a nest for an entire colony makes habitat selection an important factor for social insects such as termites. Although the arboreal termite *Nasutitermes corniger* is common to lowland and forest-edge habitats, the underlying determinants of *N. corniger* mound placement are poorly understood. In this study we surveyed mounds of *N. corniger* in Palo Verde National Park, Costa Rica, to identify factors that influence mound placement. We hypothesized that: 1) *N. corniger* mound size would be correlated with branch size, 2) larger mounds would be found higher off the ground, and 3) that more mounds would be found in early successional rather than later successional dry forest. We measured mound circumference, branch circumference, and mound height off the ground for 15 *N. corniger* mounds in both early successional and later successional dry forests ($N_{\text{total}} = 30$). We measured the abundance of *N. corniger* mounds in twenty 10m x 10m plots in each of early successional and later successional dry forests ($N_{\text{total}} = 40$). We found no evidence that mound size was related to branch size. Similarly, there was no significant relationship between height of the mound above the ground and mound size. Finally, we found no significant difference in *N. corniger* mound abundance between early and later successional dry forest. Future research should investigate the mechanisms that allow *N. corniger* to adapt to and succeed in a wide variety of habitats.

Key words: *Nasutitermes corniger*, habitat selection, Palo Verde Costa Rica

INTRODUCTION

Many social insects invest a great amount of energy and resources into the construction of their nests. Choosing a site for nest building determines access to food, and protection from predators. Considering the cost of nest building and the importance of site choice, the selection of a suitable habitat is critical for the integrity of the nest, and thus the success of the colony. Social insects often account for a large proportion of the biomass in their habitat, and play important roles in the cycling of nutrients through ecosystems. By understanding the factors that affect the habitat selection of these species, it may be possible to understand their distribution and further assess their roles and influence in an ecosystem.

The arboreal termites, *Nasutitermes corniger*, commonly build mounds in lowland wet-forest and forest-edge habitats (Lubin 1983). As termites make up a considerable amount of biomass and are essential to the recycling of nutrients, understanding the factors that influence their habitat placement may help us to understand the health of an

ecosystem. Within these habitats the driving factors responsible for nest location are poorly understood.

In this study, we expected that a combination of abiotic factors and predator avoidance strategies may influence mound site selection in Palo Verde National Park. Mound size in *N. corniger* tends to increase with colony age (Thorne and Haverty 2000). Because a greater height may increase the likelihood of survival from predation and allow mounds to grow larger, we hypothesized that mound size would be positively correlated with mound height. We further hypothesized that *N. corniger* would build larger mounds on larger branches to achieve increased structural integrity. Since *N. corniger* exhibits high survivorship and is able to relocate nests in the event of disturbances (Thorne and Haverty 2000), we hypothesized that there would be a greater abundance of mounds in early successional compared to later successional dry forest because *N. corniger* may be able to colonize disturbed areas better than competing detritivores.

METHODS

We investigated nest site selection for mounds of *N. corniger* in Palo Verde National Park, Costa Rica on 13 – 14 January 2012. We examined two habitat types and classified them as early successional and later successional dry forest. Early successional dry forest was characterized by an abundance of *Acacia collinsii* and *Guazuma ulmifolia* whereas later successional dry forest was noted for its abundance of older *Guaiaicum sanctum* and *Plumeria rubra*. To quantify *N. corniger* mound abundance in each habitat type, we surveyed the number of termite mounds occurring in twenty adjacent 10m x 10m plots ($N_{\text{total}} = 40$).

We then chose 15 mounds haphazardly from each of the two forest types and measured height above the ground from the base of each mound to the forest floor, the total circumference of the mound including the branch it rested on, and the circumference of the branch alone. We then subtracted branch circumference from the circumference of the mound with branch to obtain the actual mound circumference.

Data Analysis

We used linear regressions to assess relationships between mound size and branch size, and between mound size and height of mound above the ground. We used a two-sample t-test to measure a difference in *N. corniger* mound abundance between habitat types. All tests were conducted using the JMP 9 statistical package (SAS Institute, Cary, NC).

RESULTS

The mean circumference of *N. corniger* mounds was $41.7 \pm 4.0\text{cm}$ ($\pm 1\text{SE}$) and the mean height of mounds above the ground was $275.8 \pm 22.3\text{cm}$ ($\pm 1\text{SE}$). However, there was no

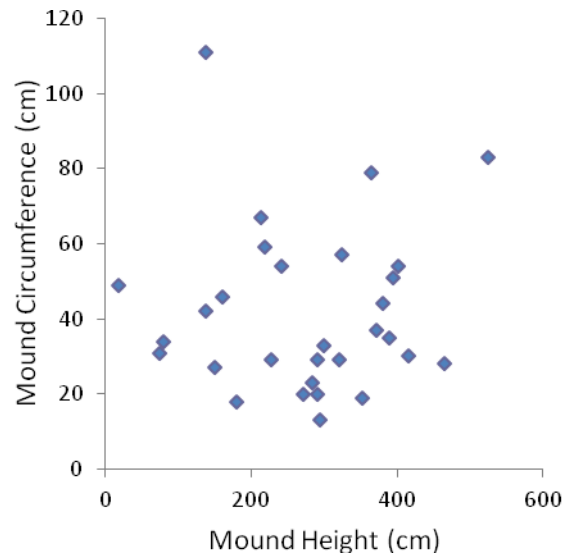


Fig 1. We found no significant relationship between mound height above the ground and mound circumference for *N. corniger* ($r^2_{1,29} = 0.01$, $P = 0.92$).

significant relationship between mound circumference and height off the ground ($r^2_{1,29} = 0.01$, $P = 0.92$; Fig. 1). There was also no significant relationship between branch circumference and mound circumference ($r^2_{1,29} = 0.04$, $P = 0.31$; Fig. 2). We found a mean of 0.5 ± 0.2 ($\pm 1\text{SE}$) mounds per 100m^2 plot in early successional plots and a mean of 0.6 ± 0.2 ($\pm 1\text{SE}$) mounds per 100m^2 plot in later successional plots, and this difference was not significant ($t_{38} = 0.42$, $P = 0.68$; Fig. 3).

DISCUSSION

In our study of termite mounds in successional dry forests, we found no significant relationship between mound circumference and mound height above the ground. This suggests that higher mound placement does not necessarily provide an advantage that reduces predation enough to allow colonies to consistently reach a greater age and size at higher heights. In fact, predation of termite nests is infrequent, mainly concentrated on mounds containing large numbers of repro-

ductives in preparation for swarming (Lubin 1983). Thus, it is possible that due to this infrequent nature of nest predation, predation pressure is not strong enough in this location to drive site selection based on height.

We found no significant relationship between mound circumference and branch circumference. We interpret this result to mean that *N. corniger* carton, the chewed wood and fecal-glue used to make nests, is strong enough to build large mounds on a variety of structures. We observed large mounds on a range of structures, from forked branches to overlapping lianas. Thorne et al. (1996) showed that termites can vary the composition and thickness of nest walls. This may allow termites a great degree of flexibility in the construction of the nest to maximize structural integrity regardless of existing branch support.

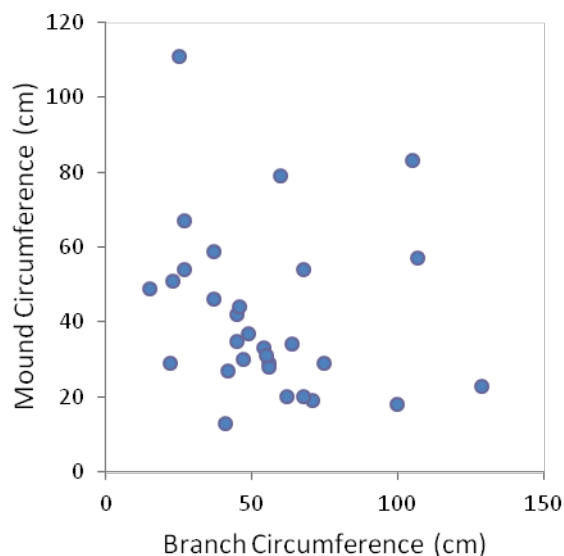


Fig 2. There was no significant relationship between branch circumference and mound circumference for *N. corniger* ($r^2_{1,29} = 0.04$, $P = 0.31$).

Finally, we found no significant effect of successional stage on *N. corniger* mound abundance per 10 m² plots. It is possible that *N. corniger* may be able to overcome such biotic differences in habitat type because it is a

successful habitat generalist. A previous study also found termites in a wide variety of habitat types, with no preferred factor driving site selection (Fonseca de Souza and Brown 1994). Although the successional habitats were different in tree species composition, critical abiotic factors for habitat selection, such as wind or temperature, may have been similar enough in both habitats to allow *N. corniger* to thrive. Future studies should attempt to better understand how *N. corniger* uses variability in mound building and placement to survive under diverse conditions.

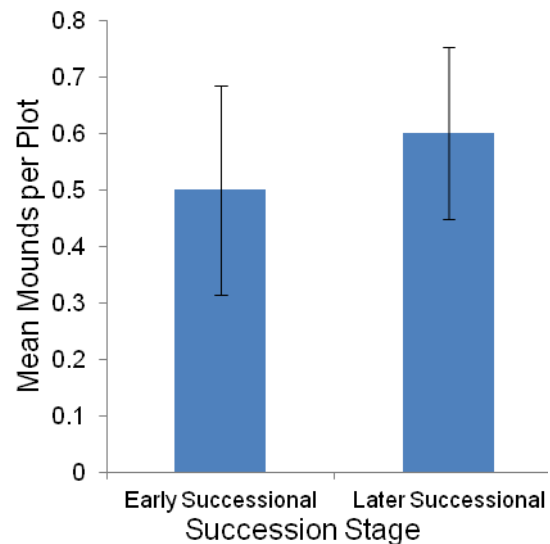


Fig 3. Abundance of *N. corniger* mounds in early and late successional forest. We found no significant effect of forest successional stage on the density of *N. corniger* mounds ($t_{38} = 0.42$, $P = 0.68$). Error bars indicate 1 standard error of the mean.

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HIBISCUS FLOWERS HAVE NO ROLE IN HABITAT RECOGNITION BY *DYSDERCUS* *SPLENDIDUS*

ANNA L. DEFFEBACH, MADILYN M. GAMBLE, BENJAMIN J. KESSLER, MILO S.
JOHNSON, AND JESSE T. RIEB

Faculty Editor: Ryan Calsbeek

Abstract: Hemipterans in the genus *Dysdercus* are agricultural pests that can decimate cotton crops. In the Guanacaste province of Costa Rica, *D. splendidus* feeds on the seeds of Turk's cap hibiscus, *Malvaviscus penduliflorus*, and previous studies have shown that *D. splendidus* is attracted to the fruit of this species. To better understand the mechanisms of habitat recognition in these insects, we investigated the role that *M. penduliflorus* flowers play in attracting *D. splendidus*. Given that flowers are a precursor to fruit, we hypothesized that there would be a positive correlation between number of flowers and number of *D. splendidus* per hibiscus. We observed *M. penduliflorus* plants in the field, and found no correlation between the presence of flowers and presence of *D. splendidus*. We used flower manipulation treatments to test the hypothesis that *D. splendidus* can use the presence of flowers to identify a suitable host in the absence of fruit. The distribution of individuals on plants following our experimental manipulations was not significantly correlated with flower presence. However, hibiscus plants on which *D. splendidus* had been found prior to manipulation were more likely to host *D. splendidus* 24 hours after manipulation, indicating that *D. splendidus* may use cues other than presence of flowers (e.g., olfactory) to locate suitable host plants.

Keywords: habitat recognition, *Dysdercus splendidus*, *Malvaviscus penduliflorus*, cotton stainer, Turk's Cap hibiscus, Palo Verde National Park, Costa Rica

INTRODUCTION

Agricultural pests cause billions of dollars in damage every year (Pimentel 2004). Many of these insects use a combination of visual and chemical cues to locate their hosts (Rommoser 1998). Thus, a thorough understanding of the mechanisms of insect host-recognition may provide a more effective means for decreasing crop damage.

Hemipterans in the genus *Dysdercus*, many of which are agricultural pests on cotton crops, are ubiquitous throughout the tropics, feeding on seeds produced by plants in the order Malvales (Bai and Koshy 2004). The adults of many species of *Dysdercus* arrive at their host plant by flight, though some disperse by walking (Derr 1980). Previous research on *Dysdercus* species has shown that the highest proportion of adults arrive at their host plant early in its fruiting stage, indicating

that the young fruits of the host plant are attractive to adults (Derr 1980). However, the exact mechanism of this attraction, whether visual, chemical, or a combination of both, is unknown.

Here, we test whether *Dysdercus splendidus*, a species occurring in the Guanacaste province of Costa Rica, uses flowers of the Turk's Cap hibiscus (*Malvaviscus penduliflorus*) as a visual or chemical cue that a plant will be a good source of these fruits and thus a high quality host. Given that hibiscus flowers are a precursor to the attractive fruiting stage, we hypothesized that more *D. splendidus* individuals would be attracted to hibiscus plants with more flowers. To test this hypothesis, we 1) examined the correlation between flower number and insect presence in the field, and 2) that experimentally manipulated flower presence and observed the subsequent change in insect presence.

METHODS

Between 09:30 and 12:30 on 13 January 2012, we haphazardly sampled 60 Turk's cap hibiscus plants between 0.5 and 3 meters in height within 10 meters of the forest edge along a road near the Organization for Tropical Studies Palo Verde research station in the Guanacaste province of Costa Rica. We marked each plant with orange flagging tape around its base and recorded the number of flowers, number of fruits, and number of *Dysdercus splendidus* present. We performed two contingency analyses with Pearson's tests to test whether the presence of flowers and presence of fruits predicted the presence of *D. splendidus*.

Later the same day between 14:00 and 17:00 we assigned 44 Turk's cap hibiscus plants to pairs such that each plant was within 8 meters of its partner. Thirty-nine of 44 paired plants had been included in our earlier observations. We pinched off all flowers and fruits and removed all *D. splendidus* by aspirator from every plant, preserving the insects and flowers for redistribution. Within each pair, we randomly chose one of the two plants via coin toss as a flower addition treatment. We added three red Turk's Cap flowers to plants in the addition treatment by wrapping flower stems in wet toilet paper and affixing them to branches with gray duct tape. We attached three pieces of duct tape to the control plants in each pair. Between 1700 and 1815, we released 2-6 *D. splendidus* individuals equidistant to either plant in each pair.

On 14 January 2012 between 14:30 and 15:30 we recorded the number of *D. splendidus* present on each paired plant. We used contingency analyses with Pearson's tests to determine if either the flower treatment or the presence of *D. splendidus* on a plant before

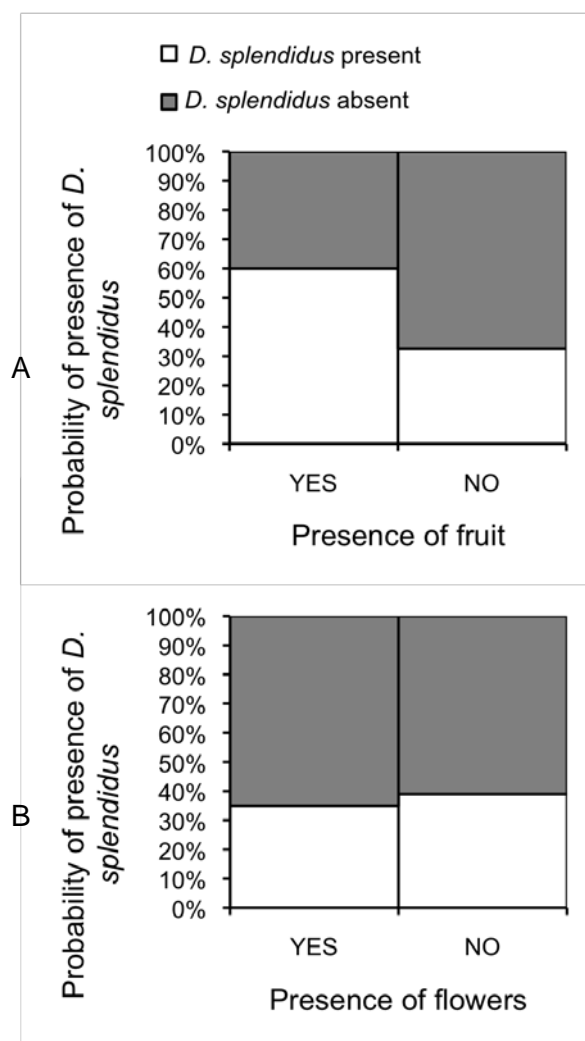


FIG 1. The presence of fruits (A) was a significant predictor of the presence of *D. splendidus* in our observational survey. The presence of flowers (B) was not a significant predictor.

manipulation predicted the presence of *D. splendidus* after manipulation. We analyzed our results using the JMP 9 statistical software package (SAS Institute, Cary, NC).

RESULTS

In contingency analyses, presence or absence of fruits (binary response) was a significant predictor of the presence of *D. splendidus* (Pearson's $\chi^2=4.15$ $P=0.042$, $n=60$), but presence of flowers was not (Pearson's $\chi^2=1.69$ $P=0.19$, $n=60$; figure 1). We found no signifi-

cant difference in total number of *D. splendidus* between flower addition treatments (Pearson's $\chi^2=0.023$, $P=0.88$, $n=39$). The presence of *D. splendidus* before plant manipulation predicted the presence of *D. splendidus* after the plant manipulation (Pearson's $\chi^2=12.17$ $P=0.0005$, $n=39$; figure 2).

DISCUSSION

As with Derr (1980), our survey of *M. penduliflorus* and *D. splendidus* suggested a strong association between host plant fruits and the presence of *Dysdercus* species. However, our analysis did not reveal a significant correlation between presence of flowers and presence of *D. splendidus*.

The addition of flowers in our experimental manipulation did not affect recolonization by *D. splendidus*, indicating that the insect does not use flowers as a cue when choosing host plants. Less than 10% of the total number of released *D. splendidus* individuals were found on manipulated plants 24 hours after the flower addition treatment, suggesting that most of the released insects may have died or found plants outside of our study site to colonize. It is also possible that the dispersal rate of *D. splendidus* is slow enough that the released insects did not have a chance to locate our experimental host plants during the 24-hour test period.

The *M. penduliflorus* that were inhabited by *D. splendidus* before manipulation were more likely to be the plants that were recolonized after manipulation. This may indicate that *D. splendidus* have the ability to determine quality of *M. penduliflorus* plants in the absence of fruits or flowers. Given that insects often use chemical pathways to identify suitable host plants (Rosomer 1998), it seems likely that these recolonized plants were higher quality hosts than the plants that did not host

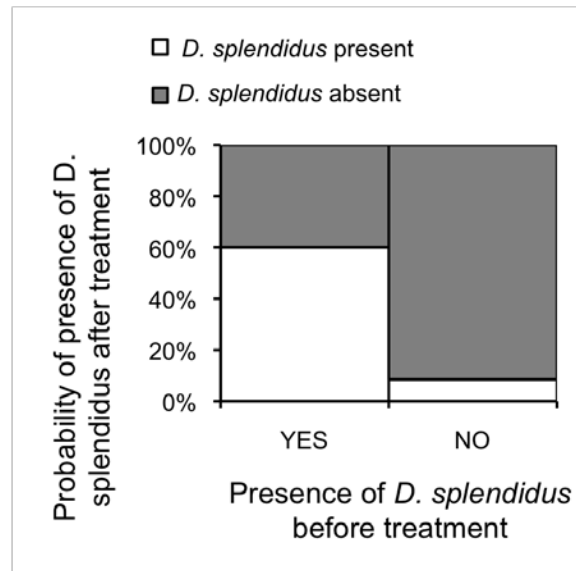


FIG 2. The presence of *D. splendidus* on a plant before treatment was a significant predictor of the presence of *D. splendidus* on the same plant after the treatment.

D. splendidus individuals both before and after flower manipulation. Future research into alternative olfactory cues used by *Dysdercus* for host plant recognition could provide useful insight into how disrupting the arrival and dispersal of insects could protect cotton crops from future infestation (Romoser 1998). Understanding the specifics of the insect-host plant relationships will aid in developing creative, economical, and sustainable methods for pest control.

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CLOUD COVER AND HERBIVORY: EFFECTS OF CLIMATE CHANGE AND A RISING CLOUD BASE ON BEGONIAS AND CATERPILLARS

ROBIN A. COSTELLO, ANNA L. DEFFEBACH, NINA B. FRANKEL, AND BENJAMIN J. KESSLER

Faculty Editor: Ryan Calsbeek

Abstract: In tropical montane cloud forests, the cloud base is rising due to climate change. Although the rising cloud base has been linked to population crashes and elevation range shifts in frogs, lizards, and birds, its effects on tropical poikilotherms and herbivory has not been tested (Pounds et al 1999). Because caterpillars are important consumers of plants, we experimentally simulated differences in cloud cover to study the effects on caterpillar growth. Because cloud contact and temperature both vary predictably with elevation, we performed our study along an elevation gradient as a proxy for understanding climate change. Along this elevation gradient we measured plant vigor (leaf water content, number of leaves, size of leaves, and plant density) and rates of herbivory on *Begonia involucrata*. Simulated cloud cover increased caterpillar growth, and the water content of leaves showed a positive trend with increasing elevation. Rates of herbivory, however, did not change over the elevation gradient. Our results suggest that climate change will disturb herbivore populations, although it is difficult to say what the effect of rising cloud cover will be on the extent of herbivory.

Key words: climate change, tropical montane cloud forest, caterpillar, elevation gradient, herbivory, *Begonia involucrata*

INTRODUCTION

Changing climate will affect ecosystems worldwide (Houghton et al. 2001). Climate change has already begun to affect tropical montane cloud forest (TMCf) ecosystems (Pounds et al 1999). TMCfs occur where tradewind-driven clouds envelop mountains, creating a low cloud base that increases moisture and lowers UV radiation (Bruijnzeel and Veneklaas 1998). TMCfs are unique ecosystems especially worth conserving as they possess high levels of endemism. They are also important for controlling local floods and erosion during the wet season and storing water during the dry season (Still et al. 1999).

In the cloud forest at Monteverde, Costa Rica, the cloud base altitude has risen due to atmospheric warming, as evidenced by a 25 year decline in both direct cloud contact and in the dry-season diurnal temperature range (Pounds et al 1999). A 2xCO₂ general circulation model of atmospheric conditions

predicts a rise in cloud base height of over 200 m during the dry season in Monteverde (Still et al. 1999). The effects of increasing cloud altitude in Monteverde have already been dramatic, contributing to synchronous population crashes in twenty species of frogs and toads and an upslope shift in the ranges of several bird species (Pounds et al 1999).

Here, we investigated the potential effects of a rising cloud cover base on herbivory. We tested the effects of changing cloud cover on a common herbivore, caterpillars, by measuring the effects of simulated cloud cover on caterpillar growth. At Monteverde, cloud cover increases along an elevation gradient while temperature decreases, allowing changes in elevation to be used as a proxy for climate change. We also explored the effects of changing climate on *Begonia involucrata*, a commonly grazed plant, by measuring herbivory levels and plant vigor (number of

leaves, leaf size, leaf moisture content, plant density) along this gradient.

According to the Resource-Defense Hypothesis, plants with better access to resources invest less in defense (Herms 1992; Stamp 2006). Therefore, in our study of *Begonia*, we hypothesized that herbivory would be greater at higher elevations due to increased plant vigor and thus decreased plant defense where there is more direct cloud contact. We hypothesized that caterpillars in simulated cloud cover manipulations would grow more than those in sunny conditions. This hypothesis was motivated by our expectation that caterpillars would grow better in conditions reflecting their natural cloudy habitat.

METHODS

We conducted our study on 20-22 January 2012 during the dry season in Monteverde, Costa Rica. To investigate the effects of climate change on herbivory, we collected 30 caterpillars of an unidentified species (individual caterpillars were characterized by mixed red, white, and black body pigmentation) at approximately 1510m in the Monteverde Biological Reserve. We weighed each caterpillar to 0.001 g. Once weighed, we placed caterpillars in individual mesh-covered cups along with a leaflet from the plant they were found on. We also provided a moist napkin to prevent leaf dehydration. We randomly selected half of the caterpillars to receive the “cloud cover” treatment, placing them in the shade of a translucent roof. The other half were assigned to a “sunny” treatment, placed one meter away in an uncovered garden which experienced changing sunlight throughout the day. We misted each “cloud cover” cup with one

ounce of water every two hours in the morning and evening and every hour from 1200 to 1400. We misted each “sunny” condition cup once at 1200 to prevent the caterpillars from drying out during the peak heat of the day. We weighed each caterpillar again after 24 hours. We then immersed the caterpillars in denatured alcohol and then put them in a drying oven (constructed from a heat lamp and cardboard boxes) for 12 hours. We re-weighed caterpillars after drying to estimate their moisture content.

To test the effects of climate change on herbivores’ host plants, we collected leaves ($n=173$) from *Begonia involucrata* over a range of elevation from 1450 to 1754 meters above sea level. We used a Garmin Oregon 400t GPS to measure elevation. Along the gradient, we selected one *Begonia* plant every 10m if one was present ($n=25$). If no *Begonia* was present at the desired elevation, we expanded our search to the 5m above and below the exact elevation. For each focal plant we recorded the number of neighboring *Begonia* plants within a 1 m radius of the stem and counted and cut off all leaves for further analysis. We then measured the total area of every leaf by tracing it on a piece of 0.5 cm grid graph paper and counting the number of graph squares within the outline of the leaf. We estimated the area removed by herbivory by counting the number of graph squares in regions of missing leaf matter. We approximated an outline of the leaf shape for regions of the leaf where herbivory damage occurred around the edge.

From each plant we selected one representative leaf to analyze for water content, excluding the largest, smallest or most damaged leaf. We weighed each leaf 6 hours after collection and put them in a

drying oven over night. We weighed the leaves again 14 hours later and recorded the percentage weight loss to estimate leaf water content.

Statistical analyses

We calculated the relative growth rate of caterpillars in the sunny and cloudy treatments using the following formula:

$$RGR = (\ln(m_f) - \ln(m_i)) / t$$

where m_f is the final caterpillar weight, m_i is the initial caterpillar weight, and t is the duration of caterpillar growth. We performed a two-sided t-test to compare the caterpillars' relative growth rates between the sunny and cloudy treatments. To determine whether differences in the amount of water absorbed by the caterpillars could account for difference in relative growth rate between treatments, we calculated moisture content by subtracting the dry mass of each caterpillar from its post-treatment wet mass. We expressed this difference as a percentage by dividing the change in mass due to water loss by post-treatment mass. We used a two-sided t-test to compare the average percent caterpillar moisture content between the treatments.

We used linear regression to compare

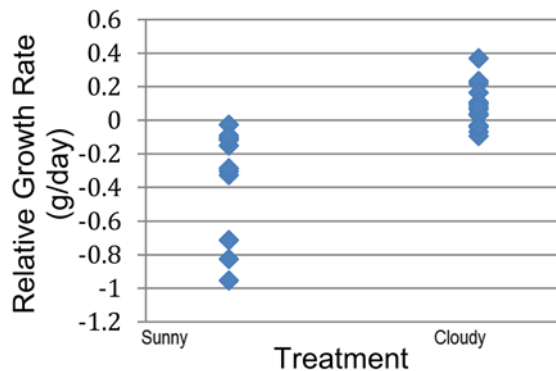


Fig. 1. The relative growth rate for caterpillars in the cloudy treatment ($0.08\text{g/day} \pm 0.13$) was significantly higher than the relative growth rate for caterpillars in the sunny treatment ($-0.36\text{g/day} \pm 0.32$).

average percent herbivory and metrics of leaf quality (water content, average leaf size, number of leaves on a plant, and number of nearest *B. involucrata*) across an elevation gradient

RESULTS

We found a significant difference in the caterpillar relative growth rate between “cloud cover” and “sunny” treatments ($t_{23,0} = -4.25$, $P = 0.0003$; Fig.1). The average growth rate for caterpillars in the cloud cover treatment (mean \pm 1SD) was $0.08\text{g/day} \pm 0.13$ and the average growth rate for caterpillars in the sun treatment was $-0.36\text{g/day} \pm 0.32$. We found no significant difference in caterpillar moisture content between treatments (student's t-test, $t_{27,8} = 1.24$, $P = 0.22$).

Water content in *B. involucrata* leaves increased with increasing elevation ($r^2_{23} = 0.15$, $P = 0.06$; Fig 2). We did not find any significant relationships between other measures of leaf quality (average leaf size, number of leaves on a plant, number of nearest *Begonia*, average percent herbivory) and elevation.

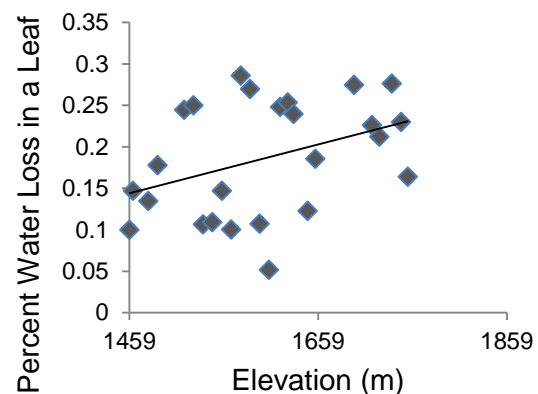


Fig. 2. Percent water loss in a leaf of *B. involucrata* increased with increasing elevation on the Pacific slope (1459-1754m) in the Monteverde Cloud Forest, Costa Rica (linear regression, $r^2_{23} = 0.15$, $p = 0.06$, $n = 25$ leaves).

DISCUSSION

Our experimental results supported our hypothesis that caterpillars would be sensitive to changes in cloud cover and grow fastest under moist and shady conditions. We showed that the difference in caterpillar weight gain between treatments was not a function of caterpillar moisture absorption, but was, rather, an accurate representation of caterpillar growth rate. In our observational *Begonia* study, we found that moisture content of leaves trended to increase with elevation. We interpret this result to mean that leaves at higher elevation provide a better food source for herbivores than to leaves at lower elevations (Mattson 1980). The increase in leaf water content with increasing elevation can be explained by increased cloud contact at higher elevations. The majority of moisture in TMCs during the dry season comes from this direct cloud contact (Still et al. 1999). Additionally, dense cloud cover at higher elevations drives nutrient uptake in plants (Leigh 1975). Reduced frequency of cloud contact driven by climate change is therefore likely to dry out the cloud forest, reducing both leaf quality and caterpillar health.

Based on the positive influence of cloud cover on both caterpillars and *Begonias*, one would expect that levels of herbivory should increase with elevation. However, our findings were not consistent with this prediction, suggesting that other ecological factors may be at play. Because different herbivore species may specialize at different elevations, there may not be a simple relationship between elevation and herbivory on leaves. Also, herbivores that do feed on *B. involucrata* may not follow the same pattern in response to cloud cover as

the caterpillars we studied here.

Alternatively, predation could put an upper limit on herbivore populations and the extent of their herbivory in the TMC (Terborgh et al 2006).

Our results suggest that caterpillars and *Begonias* are sensitive to changes in cloud cover. It is likely that many other plants and herbivores in TMCs follow these same patterns of sensitivity. If this is true, climate change will have negative implications for herbivore growth and survival in TMCs. As climate change reduces direct cloud contact, the food source quality and physiological condition will worsen for tropical montane cloud forest ectotherms.

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COSTS OF TERRITORIAL ENCOUNTERS SHAPES FORAGING BEHAVIOR IN HUMMINGBIRDS (TROCHILIDAE SPP.)

WALES A. CARTER, MUHAMMAD HEMAYAT R. CHOWDHURY, MADILYN M. GAMBLE, MADELINE K.
KREHER

Faculty Editor: Ryan G. Calsbeek

Abstract: Although Optimal Foraging Theory explains how foraging behavior should change with cost of travel between foraging sites, changes in foraging behavior in response to territoriality are not well understood. Small endothermic vertebrates such as hummingbirds (Trochilidae spp.) have extremely high metabolic rates and thus tradeoffs between the energetic costs of foraging and territorial encounters critically influence survival. In this study we explored how territorial calls affect foraging behavior in hummingbirds in Monteverde, Costa Rica. We hypothesized that: 1) hummingbirds would spend less time visiting sites where they might risk territorial encounters, 2) hummingbirds would be more willing to risk territorial encounters when the costs of travelling are greater, and 3) hummingbirds would be more willing to risk aggressive encounters if the reward at a protected site is greater than the reward at an unprotected site. We set up artificial flowers at four sites and simulated territorial calls at two of the four. We recorded the time and duration of hummingbird visits at each site before and after manipulating rewards. We found that hummingbirds spent significantly less time visiting territorial sites. Although time of day had an effect on duration of visits to control sites, duration of visits to sites with territorial calls remained constant. Finally, we found that an increased reward at sites with territorial calls compensated for the increased perceived risk of a territorial encounter.

Key words: energetic tradeoffs, hummingbird, Optimal Foraging Theory, territoriality, Trochilidae

INTRODUCTION

The cost of accessing a resource shapes foraging behavior. Optimal Foraging Theory predicts that as the costs of travel between foraging sites increase, the energy intake at the site should also increase to maintain a net energy gain (Wolf et al. 1975), but this assumes that foragers have free access to all resources. However, in territorial species, individuals protect some foraging sites, posing additional costs. Foragers who wish to access these protected resources risk energetically costly aggressive encounters and time lost foraging. Therefore, we would expect that foraging behavior in territorial species should account for the costs incurred from such encounters.

Hummingbirds are ideal organisms in

which to study the effects of territoriality on foraging behavior because they have extremely high metabolic rates for their size and must spend a fifth of the day feeding to meet these energy demands (Suarez 1992). Efficient foraging behavior is therefore vital to hummingbird survival (Fodgen and Fodgen 2005). Territorial hummingbirds protect resources with a clicking song and by aggressively chasing off other individuals, posing a cost to the foragers wishing to access protected resources (Goldberg and Ewald 1991; Fogden and Fogden 2005). Thus hummingbird foraging strategies must consider costs of territorial encounters.

We examined how the potential cost of a territorial encounter affects decisions about time spent foraging at a site. We predicted

that 1) the perceived presence of a territorial individual would deter hummingbirds from foraging at the site, 2) hummingbirds would spend more time foraging in one site and be more willing to risk a territorial encounter at mid-day when travel costs were highest, and 3) hummingbirds would be more willing to risk a territorial encounter when the reward at protected sites was greater than the reward at unprotected sites.

METHODS

On 20 January 2012 we identified four sites in which hummingbirds forage in the Monteverde Cloud Forest Biological Reserve, Costa Rica. Each site consisted of four artificial flowers within a 1m radius suspended from a tree located near the research station's manicured gardens. We constructed 16 artificial flowers by wrapping 60 ml graduated test tubes with red tape and covering the opening of each tube with a square of red paper with a hole punched through the middle to serve as a nectar access point. Between 14:00 and 15:00 we suspended each artificial flower with 50 ml of 30% sucrose solution, and allowed them to remain in place overnight so the hummingbirds could acclimatize to their presence.

On 21 January 2012, at two sites we continuously played a recording of local hummingbird calls to simulate sites defended by territorial individuals and designated these sites as "territorial". At the other two sites we continuously played a recording of songs of non-predatory bird species that do not compete with hummingbirds for resources but are common to Monteverde and designated these sites as controls. Sites were designated as "territorial" or "control" such that each territorial site was within 5m of a control site. We observed all four sites from 06:20 to 07:00, from 07:40 to 12:00, and from

13:00 to 18:00, ensuring that songs were being played throughout the observation time. At each site we recorded the time and duration of each hummingbird visit; a "visit" was defined as beginning from when a hummingbird initiated feeding from the artificial flowers and ending when it left the site.

On 22 January 2012, we increased the number of artificial flowers at the territorial sites to six (treatment "Territory-up"), reduced the number of artificial flowers at the control sites to two (treatment "Control-down"), and observed all four sites from 0800 to 1200 as before.

We performed a t-test on the mean duration of visits to determine the effect of territorial calls on the time spent foraging at a site when rewards were equal. We used ANCOVA to test the interaction between the square (ie., quadratic term) of time of day and treatment on duration of visit. To analyze hummingbird response to territorial sites where rewards were greater than at unprotected sites, we performed a t-test on the mean duration of visits to sites with

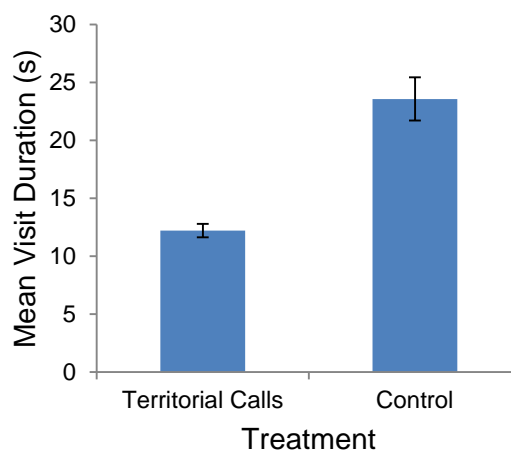


Figure 1. Mean duration of hummingbird visits to plots with and without simulated territorial calls. Hummingbird visits to control sites were approximately twice as long as visits to sites with simulated territorial calls. Bars indicate standard error.

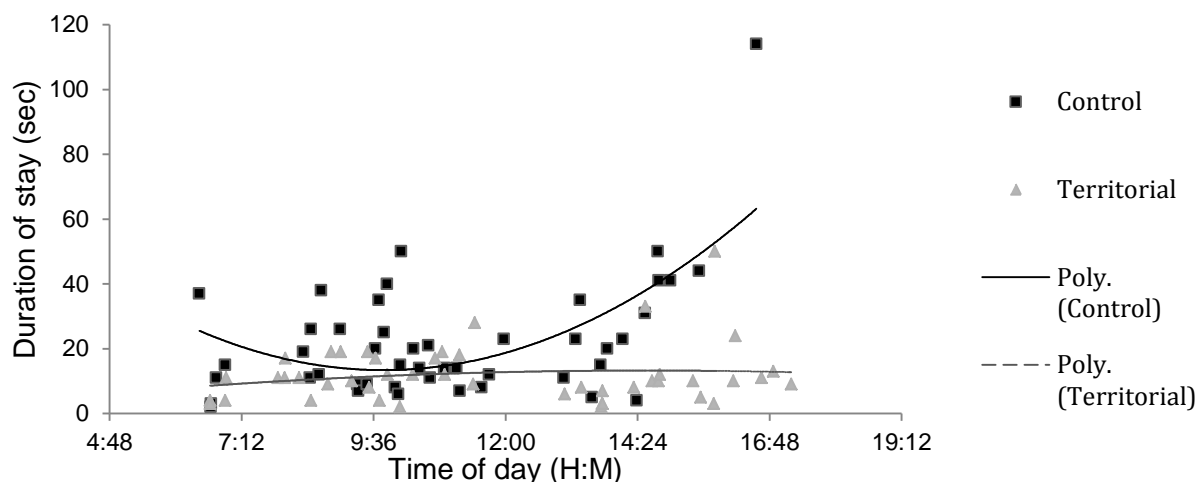


Figure 2. Duration of hummingbird visits to control sites and sites with simulated territorial calls as a function of time of day. Visits were shorter during mid-day and longer in the morning and afternoon. Hummingbird visits to sites with territorial calls did not change significantly as a function of time of day.

Table 1. Effects table of ANCOVA parameters analyzing the interaction between square of time of day and treatment with respect to duration of hummingbird visits

Source	DF	Sum of Squares	F Ratio	Prob > F
Start	1	2574.5774	15.9636	0.0001*
Start*Start	1	1399.6640	8.6786	0.0041*
Treatment	1	127.7127	0.7919	0.3760
Start*Treatment	1	1438.7043	8.9206	0.0037*
Start*Start*Treatment	1	1844.5123	11.4368	0.0011*

manipulated rewards (Cdown and Tup treatments). To compare duration of visits to territorial and control sites with manipulated rewards to duration of visits at territorial and 0800 and 1200.

RESULTS

Mean duration of hummingbird visits to control sites was approximately twice the mean duration of visits to territorial sites when rewards were equal ($t_{1,3}=5.84$, $P=0.028$; figure 1).

There was a significant interaction between the square of time of day and treatment on duration of visit ($F_{1,86}=11.44$, $P=0.001$; table 1, figure 2). Duration of stay at control sites was shorter at mid-day and longer in the morning and afternoon. Duration of stay at territorial sites was constant throughout the day.

control sites with equal rewards, we performed a t-test on the mean difference of duration of visits between days for each site, constraining both data sets to visits between

Mean duration of hummingbird visits was significantly longer at territorial sites with increased rewards than at control sites with decreased rewards ($t_{1,3}=-8.06$, $P=0.015$; figure 3). There was no significant difference between mean duration of visits to control sites and territorial sites between 08:00 and 12:00 when rewards were equal (t-test, $t_{1,3}=0.29$, $P=0.797$). With six artificial flowers at territorial sites the mean duration of visit to territorial sites was approximately six seconds longer than when there were four artificial flowers at territorial sites. When there were two artificial flowers at control sites the mean duration of visit to control sites was approximately four seconds shorter than

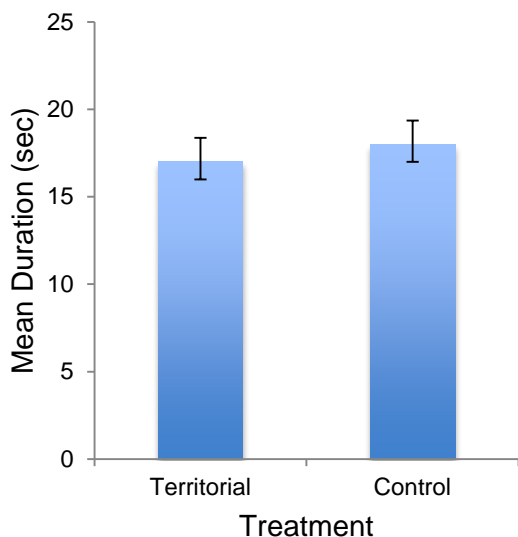


Figure 3. Mean duration of hummingbird visits to territorial sites with increased reward and control sites with decreased reward. Visits were significantly longer in territorial sites with increased rewards than control sites with decreased rewards between 0800 and 1200. Bars indicate standard error.

when there were four artificial flowers at control sites. The difference between the change in mean duration of visit to territorial sites and the change in mean duration of visit to control sites was marginally significant ($t_{1,3}=-3.82$, $P=0.0622$; figure 4).

DISCUSSION

Visits to our control sites were nearly twice as long as visits to territorial sites when nectar rewards were equal, suggesting that hummingbirds spend less time foraging in areas that appear to be defended by territorial hummingbirds. These results support our hypothesis and suggest that the potential energetic cost of a territorial encounter is powerful enough to outweigh the potential energetic benefit of foraging in a patch.

We also found that the interaction between time of day and treatment had a significant effect on the duration of visits to our artificial flowers in the absence of territorial calls, resulting in a parabolic

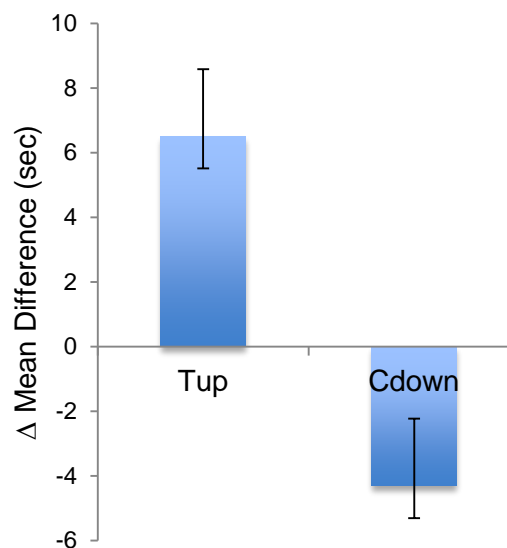


Figure 4. The difference between the change in mean duration of visit to territorial sites and the change in mean duration of visit to control sites was marginally significant between reward manipulation treatments ($t_{1,3}=-3.82$, $P=0.0622$). Tup represents territorial sites with increased rewards; Cdown represents control sites with decreased rewards. Bars indicate standard error.

function with a minimum duration at approximately 10:00. This result was not consistent with our hypothesis that visit duration would peak in the middle of the day, based on the assumption that costs of foraging and travel would also peak at that time. Since we assume that hummingbirds must constantly forage during the daylight hours to maintain energetic homeostasis, a possible explanation is that flight is least expensive in the middle of the day when hummingbirds have lower costs of thermoregulation due to higher ambient temperatures. Longer visits earlier and later in the day might be explained by an increased need for caloric intake, both to maintain thermal homeostasis and to store energy to survive the night. This mechanism is consistent with previous studies on the foraging efficiency and temperature regulation of hummingbirds (Wolf et al. 1975; Powers 1992). Another possible explanation is

that shorter visits may have been an artifact of habitual feeding patterns. Though the quantity of nectar in our artificial flowers remained nearly constant, many flowers become nectar-depleted towards the middle of the day (Fogden and Fogden 2005), and less time is required for hummingbirds to extract most of the available nectar at midday in a natural setting.

Whereas visits shortened at control sites during the late morning before rising again, duration of visits to sites with territorial calls remained constant throughout the day. These results are consistent with the explanation that the cost of an aggressive interaction remains consistently higher than the reward at territorial sites throughout the day. This suggests that hummingbird foraging strategy minimizes the risk of a territorial encounter by lowering the time spent in territorial sites, despite the changing costs of travel. Because there was a greater change in visit duration at territorial sites when we altered the reward than at control sites when we decreased reward, we feel that the increased reward is the best explanation for this trend. Because visit duration at territorial sites responded more strongly when reward was manipulated compared to visit duration at similarly manipulated control sites, we feel that the increased reward is the best explanation for this trend.

When we increased the reward at our territorial sites and decreased the reward at our control sites, we found the opposite pattern from that which was observed with equal rewards - the mean duration of visits at territorial sites was significantly longer than at control sites. The difference in these patterns between equal and unequal rewards was marginally significant. We suspect that there is a tradeoff between the risk of an aggressive interaction with a territory holder

and the rewards offered by a better patch of artificial flowers. By increasing the reward, the balance may be shifted so that the potential gain from a good patch of flowers overcomes the potential cost of an antagonistic encounter. This could then free the hummingbird to spend the optimal amount of time foraging in a patch without fear of the disruption to their energetic balance that would otherwise occur. Unfortunately one of the most important constraints on this part of our study was that data on manipulated rewards and data on equal rewards were collected on different days. However, because visit duration at territorial sites responded more strongly when reward was manipulated than visit duration at control sites responded, we feel that the increased reward is the best explanation for this trend.

All of these conclusions highlight the importance of energetic tradeoffs as a determinant of hummingbird behavior. As a result of hummingbirds' small mass and high metabolism, they have an extremely small margin of error within which they can operate (Tiebout III 1991). Hummingbirds must use a variety of behavioral mechanisms to maintain their energy balance. The behavioral responses to time of day and territoriality have the potential to play an important role in the energetic homeostasis of hummingbirds and are consistent with the predictions of Optimal Foraging Theory.

Future studies should examine other energetic tradeoffs within the framework of Optimal Foraging Theory that determine foraging behavior in hummingbirds. We used time of day as a proxy for temperature, but controlled studies of hummingbird respiration rates as a function of temperature, as well as accurate measurements of ambient temperature at each site, would provide a

direct link between energetic costs and foraging decisions. Moreover, with a greater resolution of species it may be possible to find guild- or species-specific behavioral mechanisms of maintaining energy balance. By tracking individual behavior rather than observing total activity at a site, we could draw more concrete conclusions about optimal foraging in hummingbirds. Lastly, Optimal Foraging Theory allows us to make predictions about the frequency of feeding behaviors in addition to their duration. Frequency of visits may show similar responses to time of day and territorial calls, or may respond differently as a different mechanism of energy balance. These questions must be addressed before we can fully understand the energetic costs that determine hummingbird foraging behavior.

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MOSS AND LICHEN COVERAGE CHANGES OVER A MOISTURE GRADIENT IN THE MONTEVERDE CLOUD FOREST

SHEA E. FLANAGAN AND JESSE T. RIEB

Faculty Editor: Ryan Calsbeek

Abstract: Changes in resource availability can affect both species richness and competitive relationships. To investigate these effects, we studied epiphytic communities in a cloud forest across a moisture gradient. Epiphytic mosses and lichens are abundant in the Monteverde Cloud Forest Reserve and both are limited by water availability. We hypothesized that as elevation, which is positively correlated with moisture availability, increased, both lichens and mosses would increase in richness. We surveyed richness and percent cover of mosses and lichens on tree trunks along the moisture gradient. We found that moss richness and percent cover increased with elevation, while lichen richness and percent cover decreased. This suggests a competitive relationship between the epiphytes. We also found that tree size influenced the relationship between elevation and epiphyte diversity, indicating that limited space intensified the competitive relationship.

Key Words: epiphyte, cloud forest, richness, Monteverde, moisture gradient, interspecific competition

INTRODUCTION

Ecosystems with more resources tend to be more productive, but a consistent relationship between resource availability and species richness has not been well established (Waide et al. 1999). Changes in resource availability can affect the competitive relationships between organisms within an ecosystem. All else being equal, as resource availability decreases, interspecific competition tends to increase (Wilson and Tilman 1993).

In this study, we examined the structure of epiphytic communities across a resource gradient to explore the effects of resource availability on biodiversity and interspecific competition. Though they make up a relatively small proportion of total forest biomass, epiphytes greatly impact forest processes by intercepting rainfall, providing substrates on which other organisms can grow, and fixing nitrogen from the atmosphere (Benzing 1998). Epiphyte growth, especially that of non-vascular epiphytes such as mosses and lichens, is constrained by water availability since epiphytes grow on vertical surfaces and

cannot draw water from the soil (Zotz and Heitz 2001). Epiphytic organisms compete with each other for sunlight and space as well as water.

Since water availability is the most important abiotic factor contributing to epiphyte growth (Laube 2003), we examined how the changes in water availability affect epiphytic moss and lichen community development. We hypothesized that as elevation, and therefore moisture, increased, both lichens and mosses would increase in richness.

METHODS

Study System

Our study took place along the Sendero Principal, a foot-path in the Monteverde Cloud Forest Reserve, Puntarenas Province, Costa Rica. Here, clouds pass over the ridge from east to west and release precipitation, which creates a moisture gradient of decreasing moisture down the western slope (Janowski et al. 2009). We sampled sites on the western slope's moisture gradient.

Field Methods

On 20 and 21 January 2012, we set up eight 5m by 5m plots. We sampled two replicate plots at four elevations levels along the transect, each level separated by 50m of elevation change and ranging from 1600m to 1750m. Along the transect, we measured the circumference at a tree height of 1m for each woody stem over 1m in height, and identified all moss and lichen morphotypes on the tree trunks (n=79) below a tree height of 1m. From

assigned to “small” and “large” size classes based on whether their surface area was less than or equal to the median, or greater than the median. Moss, lichen, and total species richness were determined for each tree as the number of respective morphotypes found on the tree. The moss:lichen ratio was calculated for each tree as moss richness divided by lichen richness.

We used linear regression to compare log-transformed surface area of tree trunks

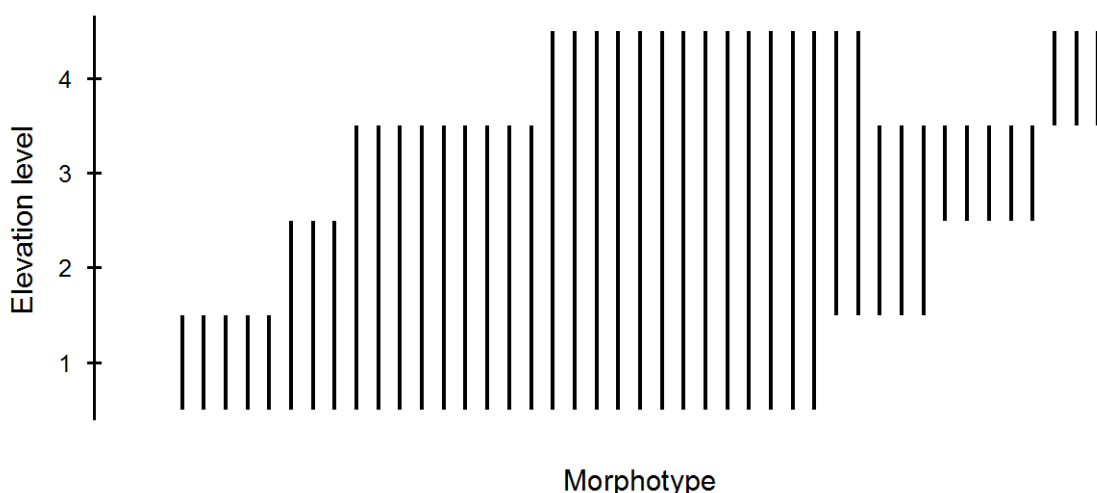


FIG. 1. Ranges of morphotypes occurring in elevation levels 1-4. Each bar represents one morphotype. Thirteen morphotypes were found at only one elevation level, 6 were found across two elevation levels, 11 were found across three elevation levels, and 13 were found across all four levels.

three randomly selected trees in each plot, we estimated percent cover of moss and lichen from the tree base to 1m along two vertical transects. We haphazardly positioned the first transect, and then positioned the second 180° from the first. To account for variation in coverage between the two sides of the tree, we averaged the values from the two transects to calculate our final coverage estimate.

Statistical Analyses

We calculated surface area in cm² as the trunk diameter (in cm) multiplied by the vertical distance measured (100 cm). Trees were

and moss, lichen, and total richness. We also used linear regression to analyze the relationship between elevation and percent coverage of moss, lichen, and bare trunk. We used ANCOVA to test for interacting effects of elevation and tree size on the moss:lichen ratio. All analyses were performed using the software package JMP 9 (SAS Institute, Cary, NC).

RESULTS

We identified 24 morphotypes of moss and 28 morphotypes of lichen. Of all morphotypes, 13 were found at only one elevation level

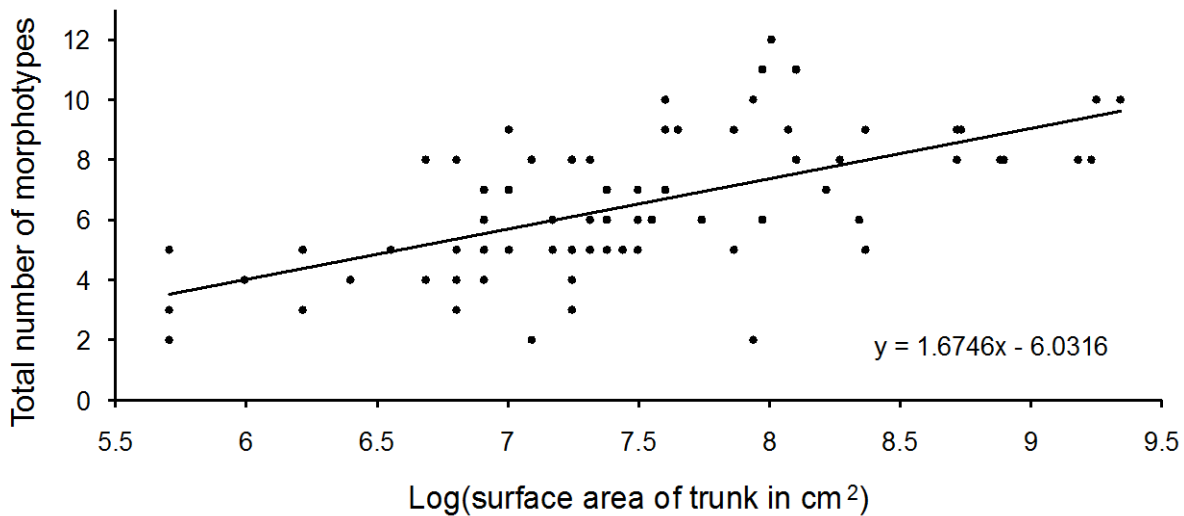


FIG. 2. Total morphotype richness increased with log-transformed tree surface area ($r^2_{79} = 0.38$, $P < 0.0001$).

(where level refers to the 50m sampling sites), 6 were found across two levels, 11 were found across three levels, and 13 were found across all four levels (Fig. 1). Morphotype richness increased with log-transformed tree surface area for mosses ($r^2_{79} = 0.14$, $P = 0.0005$), lichens ($r^2_{79} = 0.23$, $P < 0.0001$), and total epiphytes ($r^2_{79} = 0.38$, $P < 0.0001$; Fig. 2). Mean percent cover of moss increased with elevation ($r^2_7 = 0.69$, $P = 0.01$; Fig. 3). By contrast, mean percent cover of lichen decreased with elevation ($r^2_7 = 0.80$, $P = 0.002$; Fig. 3). This difference resulted in a significant interaction term in an ANCOVA contrasting elevation and epiphyte type (meaning moss or lichen) ($F_{1,44} = 57.17$, $P < 0.0001$; Table 1). Mean percent of tree trunk area with no epiphyte cover did not vary with elevation ($r^2_7 = 0.01$, $P = 0.86$; Fig. 3). There was also a significant interaction between elevation and moss:lichen abundance ratio (ANCOVA, $F_{3,73} = 10.34$, $P < 0.0001$; Fig. 4). The moss:lichen ratio increased with elevation at a greater rate in small trees than in large trees (Fig. 4).

DISCUSSION

We found that most epiphytic morphotypes ranged across at least two of the sampled elevation levels. The majority of morphotypes were found in either the highest or lowest elevation level, indicating that their elevational range is potentially greater than what we measured.

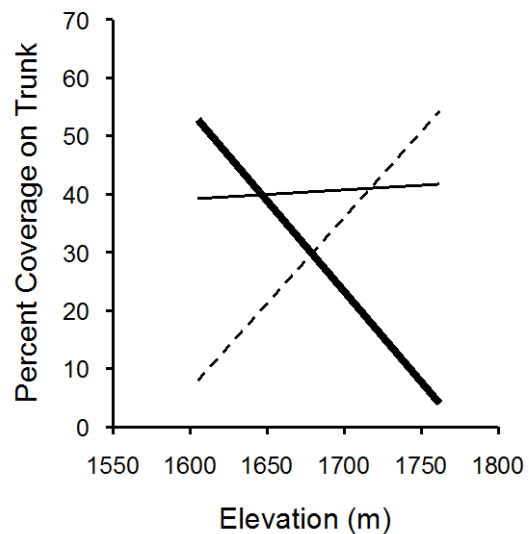


FIG. 3. As moisture availability increased, mean moss coverage (dashed line) increased ($r^2_7 = 0.69$, $P = 0.01$, $y = 0.3x - 465.6$), mean lichen coverage (heavy line) decreased ($r^2_7 = 0.80$, $P = 0.002$, $y = -0.3x + 551.7$), and the mean area uncovered (solid line) remained relatively constant ($r^2_7 = 0.01$, $P = 0.86$, $y = 0.02x + 13.9$).

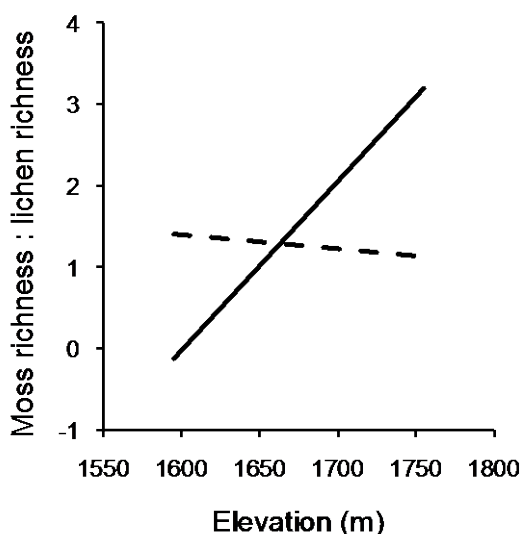


FIG. 4. The relationship between moss:lichen ratio and elevation varied with tree size (ANCOVA, $F_{3,73} = 10.34$, $P < 0.0001$). The moss:lichen ratio increased with elevation at a greater rate in small trees than in large trees.

As log-transformed trunk surface area increased, the total number of morphotypes present also increased for total epiphytic richness, lichen richness, and moss richness, following a general species-area curve. Species richness increased more rapidly at smaller areas but levels off as the number of species in an area approaches the total number of species in the ecosystem.

Both lichens and mosses grow better in higher moisture environments (Umaña and Sipman 2002), but our data showed that as elevation (a proxy for moisture) increased, lichen cover decreased, while moss cover increased. Unoccupied space on trees did not vary with elevation, suggesting that mosses and lichens may compete for suitable space. The abiotic needs of mosses and lichens vary, which could explain why the mosses and lichens varied in abundance over the moisture gradient. Though both groups lack vascular systems and rely on direct moisture, the fungal component of lichens can store water, possibly allowing lichens to better tolerate

drier conditions (Umaña and Sipman 2002). Lichens also produce allelopathic compounds that can inhibit moss germination, making it difficult for mosses to establish on lichen-covered trees (Lawrey 1986). However, mosses could overcome these defenses in favorable moisture conditions. Due to mosses' higher growth rates (Nadkarni 2000) and protruding physical structures that allow mosses to grow outwards from the tree trunk, mosses could out-compete lichens for access to sunlight.

We also found the ratio of moss:lichen species richness was related to both elevation and tree area. On the smaller 50% of trees (by circumference), more lichen species were present at low elevations, while more moss species were present at high elevations. On the larger 50% of trees (also by circumference), the numbers of moss and lichen species were approximately equal across all elevations. This indicates that the intensity of the competitive relationship between lichens and mosses varies with available area. When space is available, the two groups are able to coexist on the same tree, whereas when space is limited, the better-adapted epiphyte group tends to dominate.

Though our results suggest that mosses and lichens compete for space, our observational data cannot definitively prove this relationship. Future research could use removal and addition experiments to more precisely quantify the relationship between mosses and lichens. Investigation into other factors, such as allelopathy and competition for light, could provide further insight into the competitive relationship between mosses and lichens and the influence of space on this relationship.

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INFLUENCE OF MALE AGGRESSION ON THE SEX RATIO OF COLONIES OF A SUBSOCIAL SPIDER, *METABUS GRAVIDUS*

MILO S. JOHNSON, JAMIE E. MCLAUGHLIN, AND AMY E. VAN SCOYOC

Faculty Editor: Ryan Calsbeek

Abstract: Species that live in groups are often territorial against intruders. The relative magnitude of male aggression and female aggression often depends on the group and the particular resource being defended. For example, when males compete for reproductive resources (females), high male-male aggression is expected. *Metabus gravidus* is a subsocial spider species that lives colonially in riparian habitats. We observed 37 *M. gravidus* colonies along a river in Monteverde National Park, Costa Rica. On average we observed fewer males than females in the colonies and as such, we hypothesized that males would be more aggressive than females toward intruder males and would demonstrate male-male competition over females. Contrary to our hypothesis, we found no difference between male and female aggression towards an experimentally introduced male intruder. It appears that competition over mates is not the primary cause for the observed female-biased sex ratio. We suggest that female bias in colony sex ratios may arise due to differential dispersal success among the sexes.

Key words: aggression, male-male competition, *Metabus gravidus*, sex ratio

INTRODUCTION

Territoriality against intruders is an important characteristic of species that live in groups (Schradin 2003). Resources important to reproductive or foraging success may lead to territorial aggression (Krebs 1993). If a single sex defends reproductive resources, territorial aggression directed at intruders should be sex-specific. For example, males competing for access to females should be more aggressive towards intruding males than the females in the group. If the protection of non-reproductive resources plays a larger role, females should be just as aggressive toward the intruding male as the resident males. If true, territorial responses are expected to differ between the sexes depending on the resource being defended (Schradin 2003). Here, we investigate the difference in male and female aggression against male intruders in the colonial spider, *Metabus gravidus*.

Metabus gravidus is a subsocial orb-weaving spider that inhabits debris-laden segments of streams in Costa Rica (Buskirk

1986). By stretching their orbs across the running water, individuals are able to catch diptera and other small insects (Buskirk 1986). *Metabus gravidus* exhibit subsocial behavior; they form webs in clustered communities, but do not share food resources or exhibit other social behaviors (Buskirk 1986). It has been hypothesized that *M. gravidus* live in communities to cooperate in web building and decrease predation risk (Buskirk 1986). *Metabus gravidus* is an excellent study system for testing the difference in male and female aggression towards an intruding male, as it is possible to observe and score the reaction of the colony to an intruding male.

In colonial systems, where individuals are clustered together, males often experience a trade off between the ease of locating females and the cost of increased male competition at those sites (Uetz 1997). We observed a skewed sex ratio (female biased), leading us to investigate what factors influence the lack of males on the colonial webs of *M. gravidus*. Since male *M. gravidus* are known to mate with more than one female (R. Buskirk pers.

comm.), male-male competition for the opportunity to mate with females may lead to smaller numbers of males on the webs and explain the skewed sex ratio that we observed. We therefore hypothesized that resident males would be more aggressive towards male intruders than resident females.

To quantify the average sex ratio of *M. gravidus* colonies in nature, we surveyed webs along a stream in Monte Verde, Costa Rica. To evaluate whether aggressive interactions within colonies are based on reproductive or food resources, we experimentally introduced a male intruder into colonies, then observed and scored interactions between the intruder and resident males and females. We discuss our results in terms of the selective factors that may influence sex ratio in these spiders.

METHODS

On 21 and 22 January 2012 between 0800 and 1800, we surveyed 37 *M. gravidus* colonies along the Quebrada Máquina (Machine Creek) from the Estación Biológico Monteverde to the Hotel Belmar near Monteverde, Costa Rica. We identified all spiders visible on the web system as adult male, adult female, or juvenile. We distinguished adult males from females by leg length and abdomen shape (Alvarez-Padilla 2008; Nentwig 1993). Juveniles were too small to be accurately sexed.

We captured males from colonies that were not experimentally manipulated and placed them in separate containers. For 18 colonies of known sex ratio, we placed one 'intruder' male on a support line approximately 10 cm from the resident male. We recorded all aggressive interactions with the intruder for 20 minutes, and each interaction was given an interaction score on the follow-

ing scale: 1= close interactions with no displacement, 2 = displacement (one spider chases another away and occupies its position), and 3= fighting. We totaled the interaction scores for male-intruder and female-intruder interactions to obtain aggressiveness scores for the entire 20-minute observation in each colony.

Data Analysis

We tested the mean of sex ratios (# of males/# of females) against an expected 1:1 sex ratio. We used a Kruskal-Wallis Test to assess whether males or females had more aggressive encounters with intruding males.

RESULTS

On average, we observed a M:F sex ratio of 1:4.2, which was significantly different from 1:1 ($P < 0.0001$). Average male aggressiveness score and female aggressiveness score were not significantly different ($Z = 0.72$, $P = 0.47$, Fig. 1). Out of 18 trials, none of the intruders or resident males were completely driven off the web.

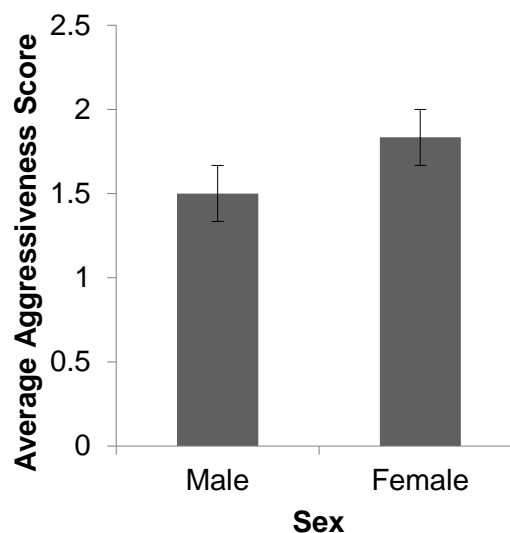


Fig 1. We found no significant difference in aggression towards a male intruder between male and female residents. Error bars represent one standard error.

DISCUSSION

The *M. gravidus* colonies we surveyed had approximately one male for every four females. If competition among males for clusters of females was the driving force behind territoriality on webs, we would expect that males would be the principle aggressors in colonies. However, resident males in our study were not significantly more aggressive towards intruding males than were resident females. In fact, we found that resident males and females expressed similar levels of aggression towards the intruder. Thus, *M. gravidus* aggression does not appear to be indicative of competition over females. Alternatively, male and female aggression may be related to other resources such as space or food. Although *M. gravidus* share common space, they still maintain individual territories within a colony (Buskirk 1975). Therefore, as male *M. gravidus* have been observed to displace both males and females from orbs, the basis for territoriality may be related to competition for space on the web (Buskirk 1975).

If colonial aggression towards intruding males induced the observed skewed sex ratio, we would have expected to see males, either the intruders or the residents, being completely exiled from the webs. In 18 trials we did not observe the colony completely drive an intruding male off the colonial web. Though aggression from individuals often drove the intruder out of the resident's immediate space, all males were allowed to remain on the web for the 20-minute observation period.

As territorial aggression did not result in the exclusion of males from the web, other explanations for the skewed sex ratio should be investigated. One possibility is that the skewed sex ratio of *M. gravidus* colonies is governed by dispersal ability and male survivorship. According to Reinhold (1996), dis-

proportionate amounts of migration between the sexes can lead to a locally biased sex ratio. Since many spider species exhibit male migratory behavior, mature male mortality is often high (Vollrath and Parker 1992). Future studies should investigate male dispersal habits in *M. gravidus*.

APPENDIX

We have included this appendix because we were surprised by how many interesting ecological questions sprung from our short observational study of *Metabus gravidus*. This subsocial species is abundant nearby the Monteverde Research Station and very easy to observe providing an excellent opportunity to study behaviors like aggression, territoriality, and group acceptance. Based on some observations, we recommend several directions for future study of these spiders. We observed a potential difference in male aggression levels depending on the relative size of the competing males. Also, during our study we marked females with whiteout and introduced them to new colonies, yet only some were observed the next day. This anomaly didn't fit into our study, but testing colony acceptance of introduced females or males over a period of a few days would be very interesting. Finally, genetic testing on the colonies to assess relatedness might shine light on the life history and dispersal of these spiders. We would highly recommend that future FSP students and researchers work with this system.

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THE EFFECTS OF TROUT EFFLUENT ON A TROPICAL STREAM MACROINVERTEBRATE COMMUNITY

M. HEMAYAT R. CHOWDHURY, SHEA E. FLANAGAN, AND JESSE T. RIEB

Faculty Editor: Matthew P. Ayres

Abstract: Nutrient availability controls the trophic structure and diversity of organisms that can coexist in a habitat. When anthropogenic activities alter nutrient availability, they can dramatically affect community structure by shifting the relative abundances of different trophic levels. To determine the effects of effluent from a trout farm on stream community structure, we sampled macroinvertebrates, both above and below the farm, in two streams at Cuericí Biological Station, Costa Rica. One stream received effluent from the farm, while the other did not. We determined that species richness and the predator:herbivore ratio increased downstream of the effluent source. The increased predator abundance suggests that the effluent addition caused a shift from bottom-up to top-down regulation in the system. Our findings suggest that up to a certain point, regulated small-scale fish farming can be practiced without negatively affecting stream ecology.

Key Words: ecosystem exploitation hypothesis, diversity-productivity hypothesis, macroinvertebrate, effluent, bioassessment, nutrient availability

INTRODUCTION

Species within a community are interconnected by biotic interactions. The strengths and relative frequency of competition, predation, herbivory, and mutualisms define community structure. Abiotic factors, such as nutrients, temperature, and dissolved oxygen, can affect the strength and direction of these relationships and thereby change the community structure. The response of communities to changes in resource availability is an important area of basic research in ecology that is also relevant to conservation, especially when considering the effects of human inputs to the environment.

There is a family of theories that seek to describe and explain the relationship between nutrient availability, diversity, and trophic structure within an ecosystem. One model, the ecosystem exploitation hypothesis, predicts that increases in nutrient availability for plants will commonly lead to increases in plant and predator biomass even while herbivore biomass remains relatively constant, due

to top-down control on herbivores (Oksanen et al. 1981). As many aquatic ecosystems are nutrient limited, resource addition tends to increase total abundance of organisms in the community (Begon et al. 1990). Also, ecosystems in the tropics tend to display positive relationships between productivity and diversity in part because of a larger species pool in the tropics (Pärtel et al. 2010).

Streams, and in particular the macroinvertebrate communities within them, tend to be responsive to nutrient loading and changes in water quality (Dudgeon 2008), so they are well suited for investigating the effects of nutrient availability on community structure. In many areas, especially in developing countries, sewage, agricultural and industrial pollution have significantly impacted the quality of stream ecosystems and shifted community structure toward more pollutant-tolerant organisms (Dudgeon 2008).

In this study we investigated how nutrient enrichment from a small-scale commercial trout farm affected the macroinvertebrate community structure in a high-elevation trop-

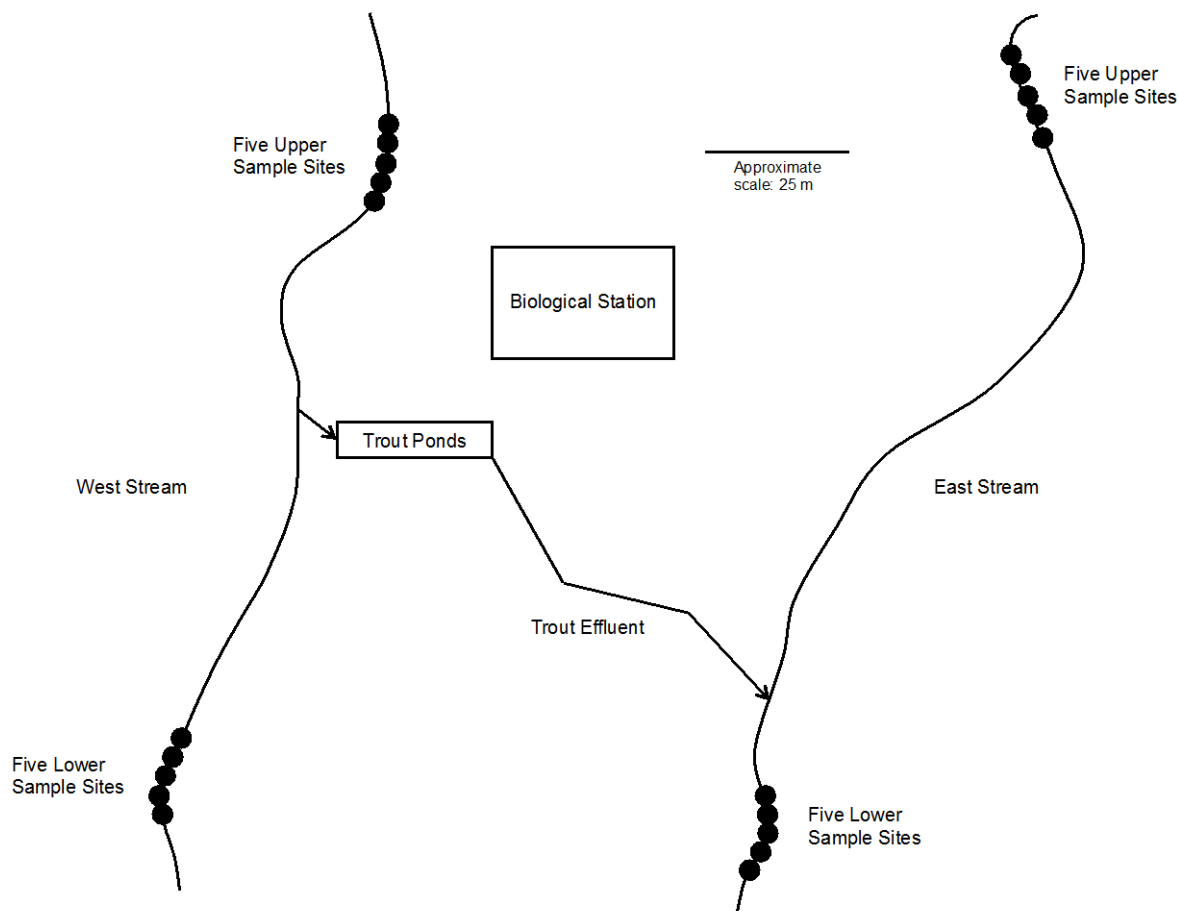


Fig. 1. A schematic of stream sample sites at the Cuericí Biological Station, Costa Rica.

ical stream. We tested whether: 1. Nutrient enrichment would lead to increased abundance and species richness of invertebrates as predicted by the diversity-productivity hypothesis, and 2. Nutrient enrichment would lead to a higher abundance of predators compared to herbivores, as predicted by the ecosystem exploitation hypothesis.

METHODS

Study site

The Cuericí Biological Station, Cerro de la Muerte, Costa Rica, offered a system to examine the effects of anthropogenic nutrient additions on stream invertebrate communities. The station is bordered on both sides by the

east and west branches of Quebrada Leones, two similar streams which come together below the station. The west branch ("west stream") is relatively unaffected by the station, except for some diversion of water, while the east branch ("east stream") receives effluent from a commercial trout farm as it passes by the station (Fig. 1). Thus, by sampling above and below the station on both streams, the effects of nutrient enrichment from the trout farm on stream communities could be separated from changes simply related to altitude. The trout effluent, which comes from a set of ponds containing at least 474 kg of rainbow trout (Costello et al. 2012), was presumed to contain copious amounts of dissolved nitrogen and phosphorus, as is true of many notable sources of water pollution (Vanni 2002).

Sampling methods

On 28 and 29 January 2012, we performed five replicate one-minute kick samples above and below the biological station (upper and lower locations) in the east and west streams. We stirred up benthic material and scrubbed rocks with our hands to detach organisms, and collected the debris with a D-net. Each sample was 2 m from the next. We measured dissolved oxygen and stream temperature at each sample location before kick samples were collected. We sorted the collected material into sampling containers with alcohol to preserve the organisms. We then identified the macroinvertebrates in each sample to order and morphotype, and identified the feeding guild and trophic level for each morphotype based on family level characteristics (Borror and DeLong 1964; Pennak 1953)

Statistical analysis

We calculated richness as the total number of morphotypes in each sample, and abundance as the total number of individuals in each sample. We also calculated the predator:herbivore ratio as the number of predator individuals divided by the number of herbivore individuals. We used two-way ANOVAs to test for the main effects of elevation, stream, and the interaction between elevation and stream on temperature, dissolved oxygen, morphotype richness, morphotype abundance, and the predator:herbivore ratio.

RESULTS

Across our samples, dissolved oxygen ranged from 6.65 mg/L (83.6%) to 7.22 mg/L (87.9%), and stream temperature ranged from 10.7 °C to 12.0 °C. The average temperature was higher in the west stream compared to the east stream (11.6 °C and 10.8 °C, respectively). The average dissolved oxygen was

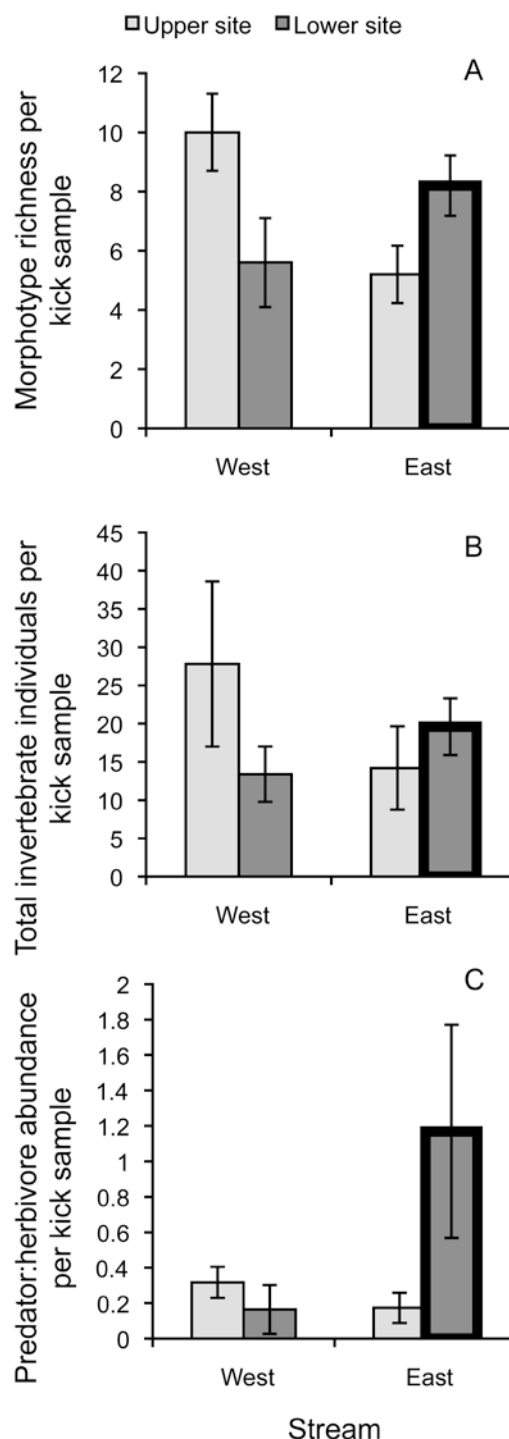


Fig. 2. Mean morphotype richness (A), mean number of invertebrate individuals (B), and predator:herbivore abundance ratio (C) per kick sample at upper and lower sites on the west and east streams. The lower site on the east stream (outlined in bold) received trout effluent. Mean \pm SE.

higher in the east stream compared to the west stream (7.07 mg/L and 6.87 mg/L, respectively). Stream identity, elevation, and their interaction were all related to stream water temperature (ANOVA, $F_{1,16} = 257.29$, $P < 0.0001$; $F_{1,16} = 24.51$, $P = 0.0001$; $F_{1,16} = 24.51$, $P = 0.0001$, respectively). Stream identity and elevation, but not their interaction, were also related to dissolved oxygen (ANOVA, $F_{1,16} = 19.69$, $P = 0.0004$; $F_{1,16} = 14.14$, $P = 0.002$; $F_{1,16} = 1.27$, $P = 0.28$, respectively).

The west stream appeared to be surrounded by larger fallen trees than the east stream, and upper sites on both streams appeared to have more canopy cover than the lower sites. There appeared to be more biomass of primary producers and a higher flow rate in the east stream than in the west stream. The most abundant feeding guild for both the west and east streams was scrapers ($n=96$ vs. $n=50$ total individuals, respectively). Of the taxa that we recognized, those with the highest overall abundance were Trichoptera ($n=109$), Copepoda ($n=94$), Ephemeroptera ($n=52$), and Diptera ($n=46$).

The average richness per sampling location decreased along the west stream by 44 percent, but increased along the east stream by 56 percent (Fig. 2A). Species richness was not significantly related to the main effects of stream location or elevation, but the interaction was significant (ANOVA, $F_{1,16} = 0.81$, $P = 0.38$; $F_{1,16} = 0.33$, $P = 0.33$; $F_{1,16} = 9.22$, $P = 0.008$, respectively). The average abundance per sampling location decreased along the west stream by 52 percent and increased along the east stream by 38 percent (Fig. 2B). The predator:herbivore abundance tended to increase along the east stream by 572 percent and decrease along the west stream by 48 percent (Fig. 2C), but neither stream location, nor elevation, nor their interaction were statistically significant (ANOVA, $F_{3,16} = 1.87$, $P = 0.19$; $F_{3,16}$

$= 1.79$, $P = 0.20$; $F_{3,16} = 3.33$, $P = 0.08$, respectively).

DISCUSSION

The trout effluent was impacting the stream ecosystem in both biodiversity and trophic structure. Although nutrient inputs frequently have negative impacts on streams, the effect in this case appeared to be positive based on the ecological criteria frequently used to judge water quality (Dudgeon 2008). The biodiversity of the west stream appears to benefit from the input of trout effluent, as indicated by higher species richness. This result is consistent with the predictions of the diversity-productivity hypothesis for streams with low productivity. As the effluent decreases nutrient limitation to plants and allows for more primary productivity, invertebrate diversity increases. However, the diversity-productivity hypothesis also predicts that, past a certain point, increased productivity can decrease diversity. For example, this could occur when high primary productivity leads to eutrophication that reduces oxygen levels (Carpenter et al. 1998). It should not be assumed that further increases in effluent input would lead to further increases in the abundance and diversity of stream invertebrates.

The fact that the effluent has increased both species richness and abundance in the stream suggests that similar tropical high elevation streams may be nutrient limited and that moderate nutrient addition via trout fisheries and/or other human activities may enhance aquatic biodiversity. This effect may similarly benefit higher trophic levels that feed on aquatic macroinvertebrates in the montane riparian ecosystem by increasing the relative predator biomass. Small tropical streams can be limited by light as well as nu-

trients, and this might explain why we did not see more conspicuous effects on macroinvertebrate abundance (Pearson and Connolly 2000). The other differences between the streams, such as variations in flow rate, allochthonous inputs, and disturbance along the streambed, might also have affected invertebrate communities by creating microhabitats, but we do not believe these noticeably altered our results.

The apparent beneficial effects of trout effluent on stream biodiversity are relevant to the fields of conservation and sustainability. If other small, high elevation streams in the area are similar in species composition and nutrient limitation, setting up small, regulated trout farms may sustainably produce trout without the undesirable downstream effects on native fauna that might be expected if ecological responses to nutrient additions were linear and uniform.

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EFFECTS OF DENSITY ON FARMED *ONCORHYNCHUS MYKISS* BODY CONDITION AND IMPLICATIONS FOR ECONOMIC SUSTAINABILITY OF SMALL-SCALE FISHERIES

ROBIN A. COSTELLO, ANNA L. DEFFEBACH, MADILYN M. GAMBLE, MADELINE K. KREHER

Abstract: High stocking density decreases the relative growth rate (RGR) of livestock by inducing competition for resources and stress responses. However, there is commonly a range of low stocking densities across which there is no effect on growth. This creates an optimal density at which farmers can maximize livestock production. We measured biomass in rainbow trout (*Oncorhynchus mykiss*) at Cuerici Biological Station to investigate the biological implications of stocking density on RGR. Additionally, we developed an economic model to make predictions about profit at different densities. Though low stocking densities produced trout with higher body conditions, the relationship was not statistically significant, indicating that the fishery is operating at or below the optimal density. Furthermore, our economic model indicated that growing trout at lower densities tends to be more economically profitable.

Keywords: intraspecific competition, *Oncorhynchus mykiss*, relative growth rate, resource limitation, stocking-density

INTRODUCTION

With limited land resources, farmers may wish to raise animals for consumption at the highest possible density. This, however, may not be the most economically profitable scheme as high densities can decrease relative growth rates (RGR) through intraspecific competition for resources or by inducing crowding stress responses (Skogland 1983; Fagerlund et al. 1981). With limited space, farmers must choose between growing many small animals and few large animals. The negative effects of density, however, do not emerge immediately. At very low densities, the addition of an individual may not limit resources per capita or elicit a stress response. Thus, low densities of livestock should not affect RGR (Fig. 1). This suggests there may be an optimum density at which a farmer produce the most biomass in the shortest time, and therefore maximize profit.

Identifying optimal densities is especially important to keep small-scale, sustainable farms viable in the increasingly competitive and chemically-altered agricultural market. However, due to the range of variables that affect this optimal density, including available food resources, life-history stages, and the

variation in size among individuals, many small-scale farmers select stocking densities based on intuition and may thus suffer reductions in profit.

To investigate the biological and economic implications of stocking density on growth rates and profit, we examined rainbow trout (*Oncorhynchus mykiss*) in a small-scale fishery at Cuerici Biological Station. We compared the body conditions of trout grown at different densities as a proxy for RGR. Bolger and Connolly (1989) found that body condition is predictive of a fish's overall health. Though RGR considers length and mass, while body condition only accounts for mass relative to length, body condition and RGR should be positively correlated.

We predicted that the body condition of trout would be poorer in ponds of greater density, indicating that the density exceeds the optimum. However, if we detected no difference in the body condition of trout among ponds, we would consider the pond under-stocked indicating that more fish could be added without negatively affecting growth.

At high densities, intraspecific competition increases. As a result, we expected a greater variance in body condition in ponds of greater densities as trout compete for limited resources. This intraspecific competition should favor larger fish, causing large trout to grow larger, and small trout to remain small. Therefore, among ponds exhibiting high variation in trout length, we expected longer fish to have a higher body condition than shorter fish.

We corroborated our biological study with an economic model to determine how varying densities and growth rates affect fishery profits.

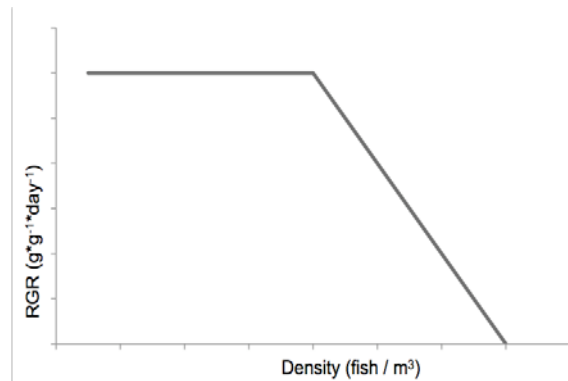


Fig 1. With increasing density, this conceptual model predicts that RGR will decrease only after the population is dense enough to cause resources to be limiting.

Study Site

The Cuerici Biological Station includes a small-scale, sustainable rainbow trout farm. Trout are raised to feed the owner's family and visitors to the Biological Station, to sell to local restaurants, and to grow to reproductive age. When the fry reach approximately 5 cm, some fish are chosen to be raised for reproduction and others for consumption. We only measured the densities of the ponds of fish being raised for consumption (ponds 4 and 10-13). The Cuerici trout farm has eight large ponds (Fig. 2). In January 2012, ponds 4 and 13

were filled with trout of the same age (one year, three months) and relatively the same size. Ponds 10, 11, and 12 contained trout of varying sizes and ages. During the study, trout received approximately a kilo of commercial fish food every day. Because the owner believed pond 11 had a lower density of trout, he fed these trout approximately a half kilo of fish food. Trout reach market size at a quarter of a kilo.

METHODS

The study was conducted on January 28 and 29, 2012. To calculate the pond water volume, we measured depth at three locations in each pond. For rectangular ponds (10-13), we also measured width at each of these three points, and obtained one length measurement. We multiplied length by width by depth to estimate the volume of water per pond. For pond 4, which was cylindrical, we measured diameter and used the formula $2\pi r^2 \times d = V$ to estimate the water volume, where r is radius, d is depth and V is volume. Additionally, we weighed the approximate amount of food given to each pond daily to the nearest 0.1g.

Using a large seine net, we collected approximately 40 fish from each pond. For each individual, we measured length to the nearest 0.5 cm from the final vertebra to the head of the trout and mass to the nearest gram. We marked fish by cutting a small triangle out of the anal fin. To recapture fish, we repeated these capture methods and noted whether fish were marked or not prior to release.

Statistical analysis

We examined the relationship between trout mass and length (Fig. 3).

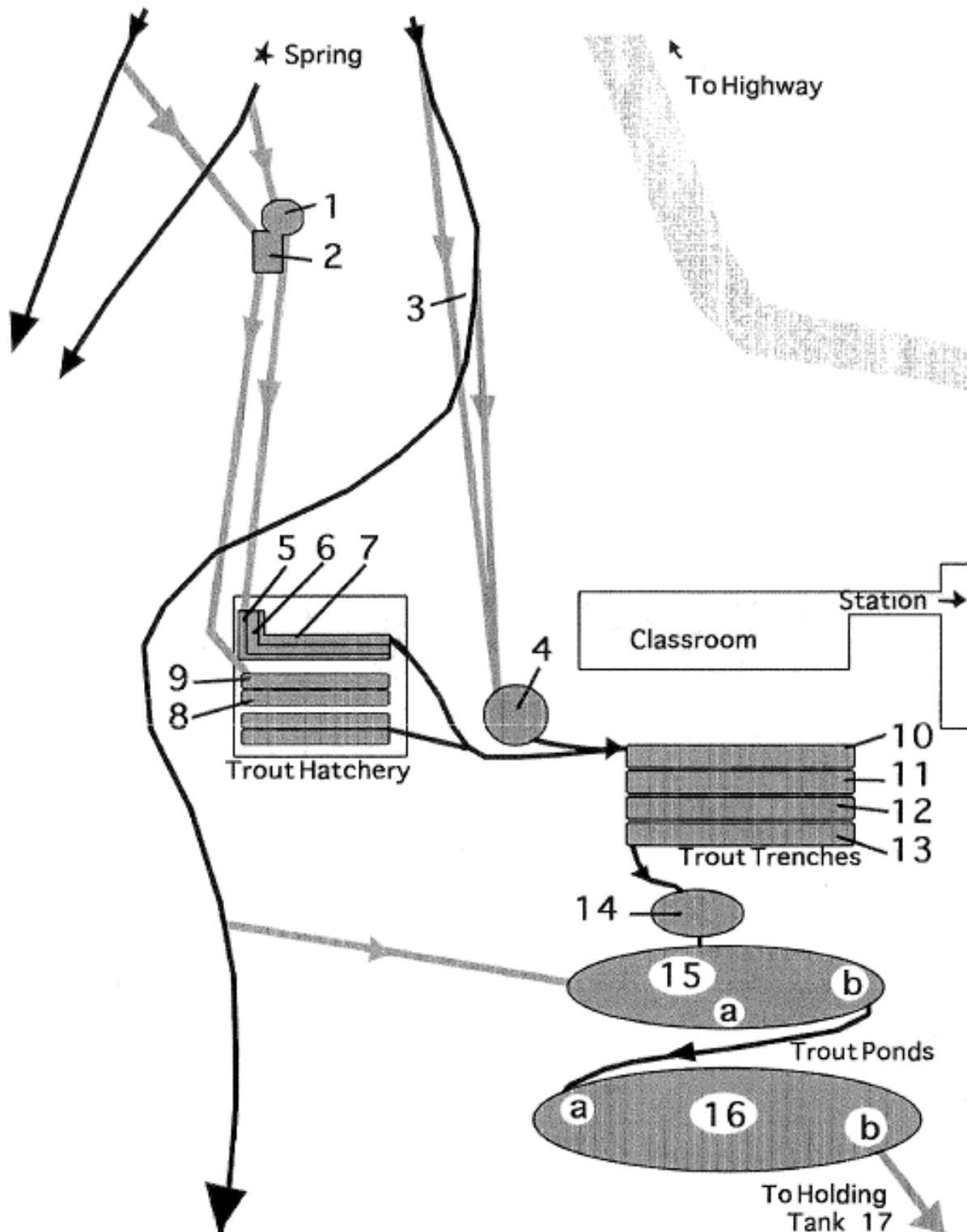


Fig 2. Map of the Cuerici trout farm. Sites 1-3 did not contain fish. Sites 5-7 contain fry raised in spring water. Sites 8 and 9 held juveniles in stream water. Ponds 4 and 10-13 contained fish being raised for commercial consumption. Pond 14 contained reproductive females that had recently spawned. Ponds 15 and 16 contained reproductive males and females. Map from Wengert et al. 2009.

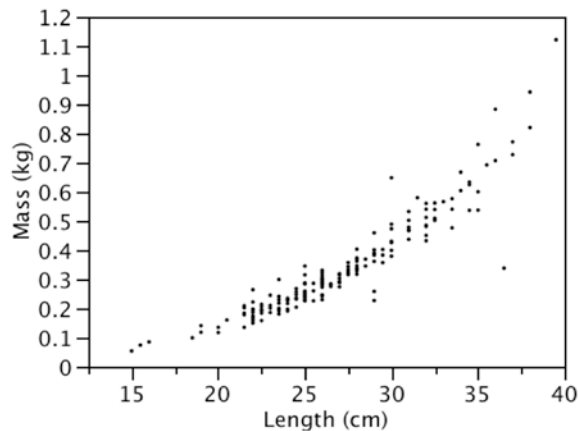


Fig 3. The relationship between length and mass in female trout.

To calculate body condition, we natural-log transformed both length and mass for female fish and fit a linear regression predicting mass from length. Residuals from this line represent body condition.

To estimate population size, we used the proportion of marked fish in our recapture as a proxy for proportion of marked fish in the total population using the following equation:

$$m/n = M/N \quad (\text{Equation 1})$$

where m is the number of marked fish in our recapture, n is the number of fish recaptured, M is the total number of fish marked in the pond, and N is the unknown total population. To calculate fish density for each pond, we divided our population estimate by the volume of water in the pond. To calculate biomass, we used the equation $(N*s)/V=b$ where N is our population estimate for the pond, s is the mean mass of the fish in the pond, V is the volume of water, and b is the biomass in kg/m^3 .

We performed two separate linear regressions with density and biomass as predictors for body condition. We also performed a linear regression with biomass as a predictor for variance in body condition.

Furthermore, we performed a linear regression with length as a predictor for body condition in ponds 10 and 11, the two ponds with the highest variance in trout length. We performed an ANOVA of body condition among low-, medium-, and high-density ponds.

Economic Modeling

To quantify the affects of changes in biomass on profit we developed a model based on RGR's reported by Bohlin et al. (2002) for wild brown trout populations in low- and high-biomass environments (Table 2). In this model, the high-biomass group contained twice the biomass of the low-biomass group. We incorporated the fishery's information on cost of food, revenue of individual fish, minimum market mass, and stocking biomasses.

We calculated time for a fish to reach market size (0.25 kg) by dividing 0.25 by RGR. We calculated revenue (R) per year with the equation $R=(5*B)*T$ where 5 dollars is the price of 1 kg of trout, B is the total biomass of the pond in kg, and T is the time it takes for a fish to reach market size. We calculated cost (C) per year with the following equation:

$$C=1.2*f*365*T \quad (\text{Equation 2})$$

where 1.2 dollars is the price for 1 kg of trout feed, f is the kg of food each pond receives daily, and T is the time it takes for a fish to reach market size. Profit (P) was defined as the difference between revenue and costs.

We performed a sensitivity analysis of the model by running 3 scenarios in which we manipulated RGR and kg of feed per day to determine the effects on profit. Since the larger trout population likely consumed more food, scenario 1 assumed that the high

Table 1. Population estimates (using mark-recapture with 2 recaptures), mean population estimate, mean density of pond, mean trout mass of pond, and mean biomass density found at the trout farm at Cuerici Biological Station.

Pond	Population Estimate 1	Population Estimate 2	Mean Population Estimate	Mean Density (fish/m ³)	Mean Mass (kg)	Mean Total Biomass (kg)	Mean Biomass (kg/L)
4	297	210	253.4	0.498	0.269	68.2	0.134
10	146	147	146.7	0.086	0.323	47.4	0.028
11	258	148	202.8	0.076	0.365	74.1	0.028
12	314	307	310.5	0.126	0.510	158.2	0.064
13	658	482	569.9	0.127	0.221	126.2	0.028

biomass environment received twice as much food (Table 2).

Scenario 2 attempted to replicate the conditions of the Cuerici fishery more specifically, where ponds 10, 12, 13, and 4 each receive 1 kg of food per day, despite differences in biomass. To account for the additional effect of resource limitation in the high-biomass ponds, we lowered the growth rate of the high-biomass fish by a conservative 0.10 kg kg⁻¹ yr⁻¹. This increases the time to reach market size by only one month (table 2).

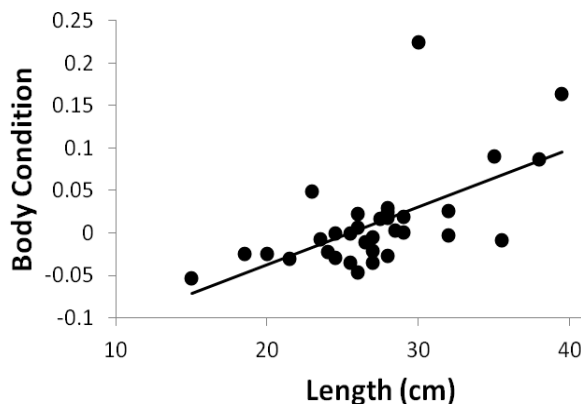


Fig 4. Body condition increased with length in pond 11, which received half as much food as other ponds.

Scenario 3 attempted to examine the economic impacts from even more dramatic differences in biomasses among ponds. The high biomass treatment was 4 times greater than the low biomass treatment, rather than only twice as great. To not overestimate the

effect of high biomass, RGR was only reduced to 1/3 of the original low biomass RGR, rather than to the 1/4 expected from a linear model (Table 2).

RESULTS

Both density and biomass varied among ponds. Density ranged from 0.076 fish/m³ in pond 11 to 0.413 fish/m³ in pond 4. Biomass ranged from 0.028 kg/m³ in ponds 10, 11, and 13 to 0.134 kg/m³ in pond 4 (Table 1).

There was no effect on body condition of stocking density ($p=0.28$, $r^2_{139}=0.008$) or biomass ($p=0.07$, $r^2_{160}=0.02$). Though ponds of greater biomass tended to contain trout of poorer body condition, this trend was not statistically significant ($p=0.12$, $F_{2,159}=2.13$; Fig. 5). Trout in low biomass ponds had a positive mean body condition of 0.012, while trout in medium and high biomass ponds had negative mean body conditions, -0.023 and -0.029 respectively.

Pools with greater biomasses trended towards less variation in body condition ($p=0.08$, $r^2_3=0.70$). However, when we looked at pools individually, we found that within the pond with the highest variance in fish length ($\sigma = 5.1$ cm), pond 11, longer fish had a higher body condition than shorter fish ($p<0.001$, $r^2_{31}=0.35$; Fig. 4). This pattern was not found in any other pond. Pond 11

received only 0.508 kg of feed, half the quantity of the other trout pond, even though the biomass of pond 11 was greater than ponds 10 and 4 (Table 1). Pond 10 had similar variation in length of trout ($\sigma^2=4.3$) to pond 11, though there was no significant effect of length on body condition in pond 10 ($p=0.30$, $r^2_{30}=0.04$).

Our economic model indicates that growing trout at a lower biomass is more profitable. Cutting the biomass of a pond in half could earn an additional US \$260 to \$400 annually for a single pond (Table 2). This would amount to between \$1300 and \$2000 in profit per year when considering the combined impact of all 5 ponds sampled. Scenario 3 emphasizes the extreme loss in profits from growing trout at a high biomass. If the biomass in even one pond is increases four-fold, annual profits would decrease by nearly \$800 (Table 2). If the biomass increased four-fold across all ponds in the fishery, profits would decrease by \$4000 dollars in a single year.

DISCUSSION

Because there was no effect of density on body condition, the trout are apparently not experiencing stress from crowding. Furthermore, the fact that there was no significant difference in body condition

between low-, medium-, and high-biomass ponds indicates that fish at Cuerici are not resource limited. This lack of resource limitation is further supported by the fact that variance in body condition did not increase with biomass of the pond, rather it trended to decrease. This suggests that there is little competition for resources in the fishery. However, since low biomass ponds had a positive mean body condition while medium and high biomass ponds had negative mean body conditions, it seems likely that biomass has some effect on body condition, and the Cuerici fishery as a whole may be near the optimal biomass represented by the growth rate model (Fig. 1). Given these results, we are hesitant to conclude whether current trout densities limit trout growth.

Though the fishery as a whole does not seem to be resource limited, there is evidence of resource limitation within pond 11. This pattern does not emerge in pond 10, though it had similar biomass and variance in length. Based on these results we believe that food is limiting RGR in pond 11, and an increase in food would result in an increase in mean trout RGR. Since RGR determines the amount of time and food that must be invested before a fish can be sold, there is a necessary tradeoff between growing many fish slowly and few fish quickly.

Table 2. Inputs and results of our economic model. In every scenario, the pond with less biomass resulted in greater profit.

Scenario	Biom- ass (kg)	RGR (kgkg ⁻¹ yr ⁻¹)	Time (yr)	Marke- table Mass (kg)	Reven- ue (\$)	Annual Reven- ue (\$)	Daily Food (kg)	Daily Food Cost (\$)	Total Cost (\$)	Profit (\$)	Annual Profit (\$)
1	100	0.45	0.56	0.25	500	892.86	2	2.4	490.56	402.30	718.39
	50	0.60	0.42	0.25	250	595.24	1	1.2	183.96	411.28	979.23
2	100	0.36	0.70	0.25	500	714.29	1	1.2	306.60	407.69	582.41
	50	0.60	0.42	0.25	250	595.24	1	1.2	183.96	411.28	979.23
3	200	0.20	1.26	0.25	1000	793.65	1	1.2	551.88	241.77	191.88
	50	0.60	0.42	0.25	250	595.24	1	1.2	183.96	411.28	979.23

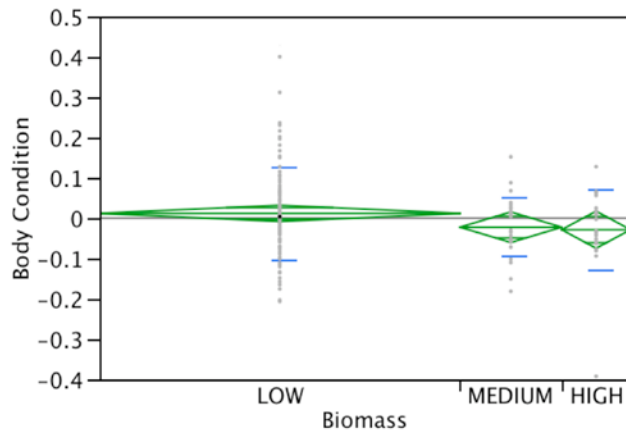


Fig 5. Trout body condition was positive in pools with low biomass per unit volume (body condition = 0.012), negative in medium biomass pools (body condition = -0.023) and negative in high biomass (body condition = -0.029).

In all scenarios of our economic model, growing fish at a lower density was the more profitable strategy. These economic predictions are only loose generalizations of the financial implications of growing trout at different densities. Though it is clear that density can have a powerful effect on profit, a more accurate model would employ growth rates measured directly from the fishery's trout populations. This would allow the fishery to make more precise decisions on how to manage trout densities to maximize profit and continue sustainable fishery practices.

ACKNOWLEDGEMENTS

We would like to thank Carlos Solano for his hospitality, patience and enthusiastic assistance.

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FACTORS INFLUENCING DISTRIBUTION OF *XYLEBORUS* BEETLES AND LEPIDOPTERA LARVAE IN *CHUSQUEA LONGIFOLIA* INTERNODES

MILO S. JOHNSON

Faculty Editor: Matt Ayres

Abstract: Resource density can influence the distribution of herbivores and their predators, depending on the relative strengths of the forces at play. Resource limitation or reproductive constraints may lead herbivores to aggregate in high-resource areas despite an increased predation risk, or the increased predation risk may cause prey species to seek partial refuge in low-resource density areas. I examined the distribution of two bamboo-boring insects, a *Xyleborus* beetle and a lepidopteran larvae, at plots with low and high densities of their host plant, *C. longifolia*. I also sought to quantify predator presence for the *Xyleborus* in each plot. I found similar densities (attacks per culm) of both species in plots with different bamboo densities, and found only one predator in twenty dissections of *C. longifolia* internodes with *Xyleborus* entry holes. These results did not indicate herbivore aggregation or partial refuge from predation, but I observed a significant dissociation between *Xyleborus* and the Lepidoptera larvae, suggesting that the two species may be in competition for suitable *C. longifolia* internodes.

Key words: aggregation, partial refuge, interspecific competition, *Chusquea longifolia*, *Xyleborus*, bamboo beetle

INTRODUCTION

The distribution of organisms is a fundamental, dynamic component of ecosystems. For example, primary producer density within habitats is a driver of consumer distribution patterns. Herbivores may aggregate in high-density patches based on habitat location mechanisms, for reproductive opportunities, and/or for future benefits for their offspring (Van Baalen 1993). If the herbivorous prey aggregate, their predators may follow suit, aggregating in high-density patches to maximize their foraging rate (Van Baalen 1993). Since high-density patches can also result in an increased risk of predation, low-density patches may serve as partial refuges where the prey may escape predation (Begon 1990). Examining which of these patterns, if any, a species follows can illuminate the relative importance of various forces potentially influencing distribution. These forces are likely a combination of evolutionary pressures influencing habitat selection behavior and

direct pressures that cause habitat choice to change, even within an organism's lifetime. If predation is a central force, prey may escape in space to partial refuges with low plant density. This leads to an even distribution across different resource densities. If resource limitation is a more significant force, herbivores should aggregate in high-density patches despite increased predator presence. For herbivores, the trade-off between resource availability and increased susceptibility to predation in high-resource-density areas makes habitat selection a complex and all-important game.

Chusquea longifolia is a high altitude bamboo species. In the Cerro de la Muerte region of Costa Rica, I found two species of insect that burrow in *C. longifolia* internodes: a species of bark beetle (*Scolytinae*; photos 1-2), and a lepidopteran larvae (photo 3). These diminutive burrowers create small-scale habitats within the bamboo culms, gaining both shelter and nutrients from the plant. I observed that both species are subject to predation or parasitism, respectively, and

their antagonists likely specialize on infiltrating their internode habitats to find meals. Since both species develop within the internodes, parents must select suitable internodes for their offspring, perhaps balancing the benefits of aggregation against the potential costs of higher predation rates in high-density areas.

Xyleborus and the lepidopteran species both leave external evidence (entrance holes or scars) on the culms, indicating the location of their galleries. This made it relatively easy to quantify where and at what density (per culm) these organisms had attacked individual *C. longifolia* internodes. I sought to investigate how the distribution of *Xyleborus* and the lepidopteran species were related to host density. I considered two competing hypotheses: 1) these burrowing insect species aggregate in high-density patches despite higher predation risk, or 2) specialist predation in *C. longifolia* exerts a strong pressure against aggregation in high-density patches, leading to an even distribution. To test these hypotheses, I sampled the number of *Xyleborus* and Lepidoptera attacks per culm in high and low density *C. longifolia* plots, and dissected *Xyleborus* internodes from each plot to quantify predation.

METHODS

I surveyed *C. longifolia* culms from 6 plots (each 9 m²) in a high altitude oak and bamboo forest in Cerro de La Muerte. I chose 3 high-density (>4.44 culms/m²) and 3 low-density (<3.33 culms/m²) plots within 100 meters of each other. I randomly selected 10 culms in each plot and examined the first 8 internodes for *Xyleborus* or Lepidoptera attacks, distinguished by the shape of the entry hole (~2 mm circular hole for *Xyleborus*, variable but much larger and vertically elongated hole

for Lepidoptera). I counted each Lepidoptera entry hole as a separate attack but counted multiple *Xyleborus* holes in one internode as only one attack, assuming extra holes were exit holes from one gallery. I measured diameter at breast height (DBH, ~1.5 m) for each culm by sight using a ruler and measured culm health on a 0-5 scale: 0 – dead; 1 - dying, culm with little turgor, dead branches or tip, no leaves; 2 - culm with few leaves, weak turgor; 3 - culm with leaves, average turgor; 4 - culm with strong turgor and leaves at most nodes; 5 - culm with strong turgor and abundant leaves at nearly all nodes.

I cut 5 internodes (only from internodes 3-5, measured from the ground up) with *Xyleborus* entry holes from haphazardly selected culms within the plots. I dissected each internode and recorded the organisms found inside. For one high-density and one low-density plot (plots 2 & 3), I could not find enough culms with *Xyleborus* attacks, so only four plots were included.

Because DBH covaried with both culm health and beetle attack rate, I used culm health residuals to remove the potentially confounding effect of DBH from correlations involving attack rates and culm health.

RESULTS

I found 6 species of insect inside the *C. longifolia* internodes: a species of bark beetle tentatively identified as *Xyleborus* (photos 1-2, pers. comm. Maria J. Lombardero, Universidad de Santiago de Compostela), a lepidopteran larvae (photo 3), a dermapteran species (photo 4) a diptera larvae (photo 5), a mirid species (Hemiptera, photo 6), and a koinobiont on the Lepidoptera larvae (photo 3). The dipteran larvae is predatory and was found in beetle galleries, both with and

without beetles. I was not able to identify the koinobiont.

Only the *Xyleborus* and the Lepidoptera larvae appear to tunnel in the bamboo, and the two resulting scars were easily distinguishable. The Lepidoptera larvae tend to make vertically-skewed oval entry holes and burrow downward along the periphery of the culm to the node. The two living larvae I found in preliminary dissections both had koinobionts on top of the fourth segment (photo 3).

The *Xyleborus* beetles make circular entry holes approximately 2 mm wide in *C. longifolia* internodes, usually just above the V-shape of the culm sheath but sometimes at the node itself (photos 7-8). The adult beetle then burrows upward in a spiral, counterclockwise when viewed from above (photo 9). I often found several adult beetles in a gallery, presumably siblings still feeding in the bamboo before dispersal and reproduction. The *Xyleborus* are winged, but I did not observe flight.

I observed several inquiline in the *Xyleborus* galleries: solitary predatory diptera larvae, groups of mirids, and groups of dermapterans (photos 4-6). Galleries with inquilines usually were hollow within the spiral but still had dark markings from the spiral burrowing pattern. In one gallery I found a hollowed carcass of a *Xyleborus* along

with a group of mirids. This may have been a female that died after making her burrow and laying eggs in the internode.

I found no difference in the number of bark beetle ($Z=-0.22$, $P=0.82$) or Lepidoptera larvae ($Z=0.31$, $P=0.75$) attacks in the first eight nodes of *C. longifolia* shoots between high and low density sites (Fig 1). Of the twenty internodes with beetle attacks that I dissected, only one internode from a high-density site had a predator, a diptera larvae, and no internodes from low-density sites contained predators. I found failed, active, and abandoned beetle galleries with dermapteran inquilines in internodes from both high and low-density sites.

I found one internode that was attacked by both Lepidoptera and *Xyleborus*, 45 internodes attacked by Lepidoptera but not *Xyleborus*, 64 internodes attacked by *Xyleborus* but not Lepidoptera, and 370 internodes with no attacks. This was significantly different than would be expected under the assumption that attacks are independent (expected values respectively: 6, 40, 59, 375, $\chi^2=5.62$, $df=1$, $P=0.018$).

After taking residuals to control for the effect of DBH, there was a significant negative correlation between number of beetle attacks per culm and culm health (Spearman $\rho=-0.27$, $P=0.038$, Fig. 2), while Lepidoptera attacks did not correlate with culm health (Spearman

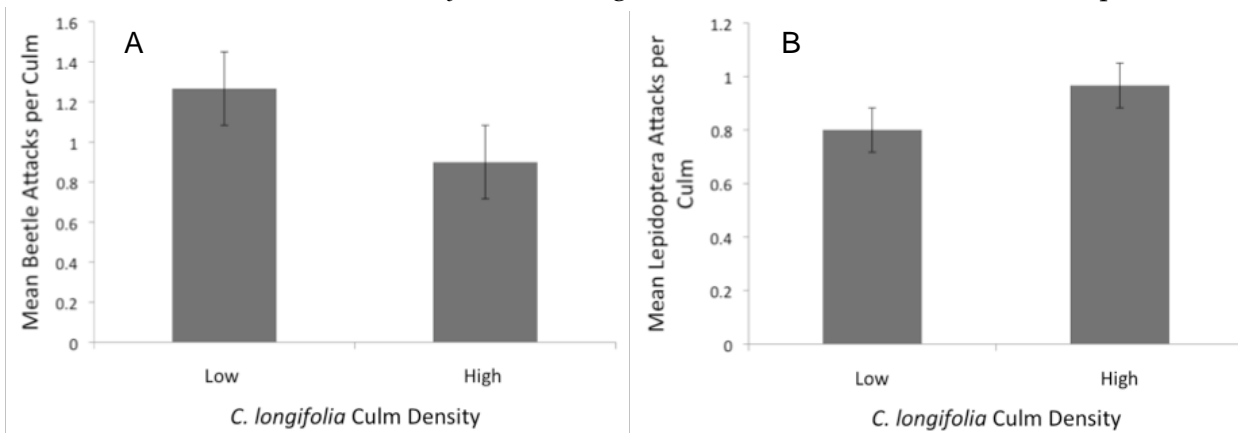


Fig. 1. The mean number of (A) bark beetle and (B) Lepidoptera larvae attacks in the first eight nodes of *C. longifolia* shoots between high and low density sites. There was no significant difference between densities. Error bars represent one standard error.

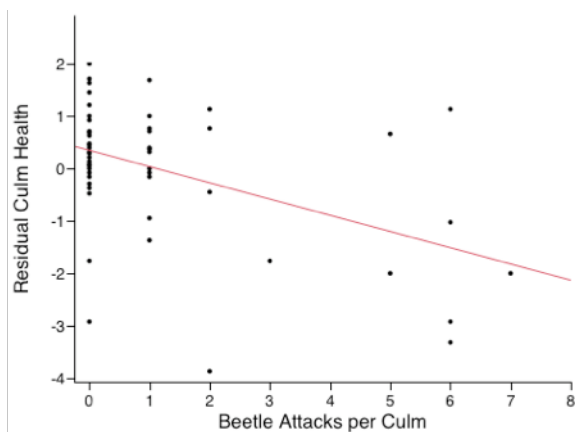


Fig. 2. Beetle attacks per culm (in the first 8 internodes) and residual culm health (to control for the effect of culm diameter).

$\rho=0.08$, $P=0.57$).

DISCUSSION

My investigations required elucidating the basic natural history of the system. As the *Xyleborus* beetle burrows into the bamboo culm and begins its spiraling journey upwards, it creates a habitat not only for its offspring, but also for predators (like the diptera larvae) and inquilines (the dermapterans and hemipterans). While the Lepidoptera larvae also carves into the culm, its burrows are usually dry and exposed once it is gone, and the only interacting species I observed was the koinobiont attached to and feeding upon the larvae. The two burrowers have different effects on the plants. The beetles, like their Scolytid relatives in the United States (White 1983), appear to have a negative effect on culm health (Fig. 2), which is not surprising based on their large interior galleries (photo 9). The Lepidoptera, who carve out a feeding gallery only on the periphery of the culm, do not have such a negative effect.

Spatial patterns of *Xyleborus* and Lepidoptera larvae distribution did not

support density-dependent aggregation. I found no difference in *Xyleborus* or Lepidoptera attacks per culm between high and low density areas, which could indicate that low-density areas are partial refuges for these species. However, the low frequency of predators in the beetle internodes does not support this hypothesis. Because I did not dissect internodes with Lepidoptera attacks, further study on the distribution of the koinobionts parasitizing the Lepidoptera larvae is needed to determine whether the Lepidoptera are seeking partial refuges. Furthermore, the scale at which I collected data deserves consideration. Habitat selection can occur at many different scales, and the dynamics will be influenced by dispersal range, which is unknown for both species in this study (Stamps 2009). Since both species were winged, dispersal ability may be high, so larger patches that are further apart and span more variable levels of density may be more appropriate for answering this question.

I found fewer internodes with both Lepidoptera and *Xyleborus* attacks than would be expected if they were distributed independent of each other, suggesting that interspecific competition may be an important force affecting the distribution of both species. Depending on the conditions that make an internode suitable for inhabitants (another avenue for further research), competition for internodes, as a source of both food and shelter for offspring, may be strong. The rarity of internodes attacked by both species indicates that if competition is at play, its primary component is the race to colonize suitable *C. longifolia* internodes, because once an insect has colonized the node, the other rarely invades. Alternately, the dissociation between the two species could be an example of resource partitioning along some gradient I did not measure, indicating that interspecific

competition has played a role in the evolution of these species. Removal or addition studies are necessary to investigate the importance of competition. Overall, my results suggest that the forces influencing distribution of the *Xyleborus* and Lepidoptera species may not stem from the availability of resources or foraging patterns of predators, but instead from a struggle between the parents of each species, who must search for culms to find suitable and unoccupied homes for their offspring. For insect species in discrete habitats like bamboo internodes, competition for food and shelter may outweigh the effects of resource density on their distribution.

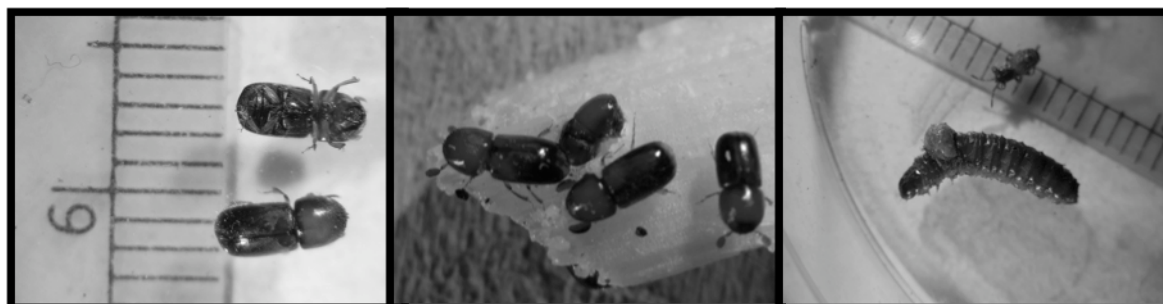
ACKNOWLEDGEMENTS

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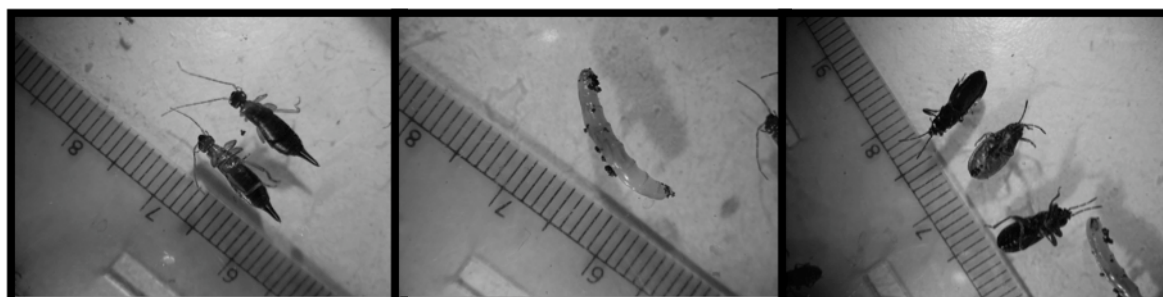
APPENDIX 1. SUPPLEMENTARY PHOTOGRAPHS



1. *Xyleborus* beetle

2. *Xyleborus* beetle

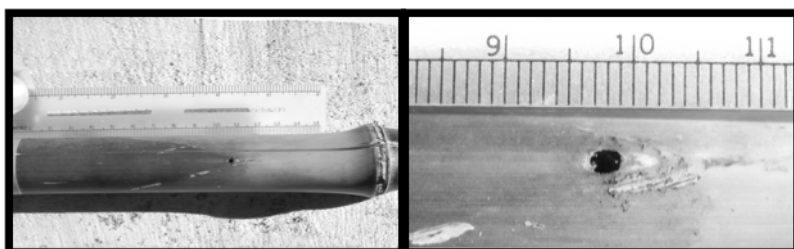
3. Lepidoptera larvae with koinobiont



4. Dermaptera

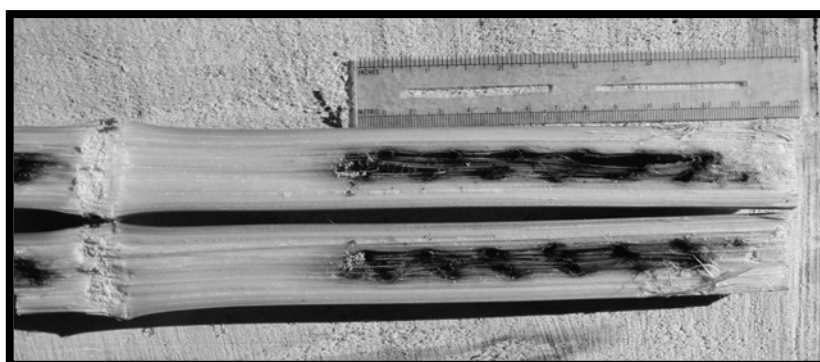
5. Diptera larvae *

6. Hemiptera



7. *Xyleborus* entry hole

8. *Xyleborus* entry hole



9. *Xyleborus* gallery in *C. longifolia*. Left is toward the base of the culm.

*The head cap, withdrawn in this photo, is free to move within the outer translucent layer of the larvae and can slide forward past of the outer layer.

All photographs courtesy of Professor Matthew Ayres.

INSECT VISITATION AND THERMAL REWARD THEORY

WALES A. CARTER, NINA B. FRANKEL, BENJAMIN J. KESSLER, JAMIE E. McLAUGHLIN,
AND AMY E. VAN SCOYOC

Faculty Editor: Matthew Ayres

Abstract: Ectotherms have a limited ability to control their body temperature and are greatly affected by their surrounding thermal environment. Temperature may therefore affect mutualisms between plants and their insect pollinators. Flowers, due to their morphology, are able to warm the air above their surface. This warming may provide an unusual pollinator-incentive system, a “thermal reward.” To investigate the existence of a thermal reward we examined how flowers in a high alpine environment of Costa Rica affected the temperature of visiting Chrysomelidae beetles and whether their behavior corresponded with this warming. Beetles became warmer inside a flower than out and beetle visit duration corresponded to the duration over which thermal benefits occurred. These findings shed some light on the role of temperature and physiology in mutualistic relationships.

Key words: Ectotherm, *Bellis perenis*, *Chrysomelidae*, mutualism, thermal reward, *Zantedeschia aethiopica*.

INTRODUCTION

The rates at which fundamental enzymatic reactions complete metabolic functions are influenced by temperature (Hochachka and Somero 2002). An ectothermic animal is one that has a limited ability to control its body temperature through metabolic heat production (endothermy). They may still remain within an appropriate temperature range by behaviorally altering their location in the environment. (Huey 1989) However, high altitude environments with high diurnal amplitude may pose an especially challenging environment for ectotherms.

Mutualisms, interspecies relationships with reciprocal benefits, could be particularly important in harsh environments. One of the most widespread and ecologically important mutualisms is that which exists between plants and their animal pollinators. Though there are many types of pollinators, the most abundant, and frequently the most important pollinators are insects. It is well known that the archetypical plant can offer nectar or other

resources as a reward to influence insect activity and entice them to transport their pollen, there can also be other mechanisms by which flowers can attract potential pollinators.

Due to the shape of many flowers, the air above their face is warmed. This phenomenon has been explained as a way to warm the reproductive organs of the flower and increase their rate of seed development (McKee, 1998). We hypothesized that this warming may have an additional purpose, playing a role in a special type of pollinator-incentive system, a thermal reward (See Box 1). Thermal rewards could enhance the plant-pollinator mutualism by providing an additional fitness benefit to both involved parties.

For thermal rewards to influence plant-animal pollinator mutualisms, the warming above a flower must affect the body temperature of a visiting insect. This change in insect body temperature must be mutually beneficial by creating a change in behavior that increases both insect and flower fitness. For example, as an insect is warmed to a favorable

temperature, allowing it to fly and forage for its nectar and pollen food sources through more hours of the day. This increased foraging activity could also enhance the fitness of the plant by increasing pollination.

Box 1: Thermal reward theory
Postulate 1: A flower has a thermal effect on visiting pollinators.
Postulate 2: The pollinator receives a benefit from this thermal effect.
Postulate 3: This thermal effect changes the behavior of pollinators.
Postulate 4: This changed behavior increases the fecundity of the plant.
Therefore, a thermal reward is present (and will be selected for).

To test for the existence of a thermal reward, we studied a high altitude insect (Coleoptera: Chrysomelidae) and two plant species of differing flower morphologies that it frequently visits. We investigated how these flower types affected the temperature of visiting Chrysomelids and whether the behavior of Chrysomelids corresponded to this warming as predicted if the flowers were offering a thermal reward.

METHODS

Our study took place on 28-30 January 2012 at Cuerici Biological Station, Costa Rica. This site experiences daily shaded air temperatures that typically range from about 9 to 18 °C. It is common for the warmest temperatures to occur in the late morning with clouds rolling in around midday and resulting in a cooler afternoon. We hung a HOBO temperature logger to collect shaded ambient air temperature over the entire course of our study. We compared the thermal effects of *Zantedeschia aethiopica*, the calla lily, and *Bellis perenis*, a common daisy; two common, white,

and perfect flowers in the area. *B. perenis* had open, flat corollas with thin white petals around a yellow central disk, while *Z. aethiopica* had a long tubular corolla with large fused petals and a protruding stamen. Both flowers had similar coloration. The insect visitors we measured were Chrysomelids (photo in Appendix A), chosen because they were frequent visitors to both flower species. We believe that these insects are pollinators and not just visitors of these two flower species because of the large amounts of pollen that typically coated their bodies upon leaving both flowers. Between 1300 and 1600 on 28 January, we captured two Chrysomelids and gently inserted fine-gauge thermocouple needles into their thoraxes (one attached to a BAT 12 and the other a HOBO data logger). At 30 seconds for 30-minute bouts, we recorded the operative temperature of these beetles when held inside and outside of a flower. We switched the beetles (with their thermocouples) at 15 minutes to control for possible differences between the two. We repeated this twice for each flower type with new beetles each time, for a total of 4 trials.

On 29 January, we tested the beetles' operative temperature in each flower type. Starting with two beetles (instrumented with thermocouples) that were at equilibrium with ambient shade temperature, we moved one into a flower in the sun and one into the sun about 50 cm away but without a flower and recorded their temperature every 5 seconds for 5 minutes. We repeated this 4 times for each flower, allowing the beetle at least 3 minutes to return to equilibrium with the ambient shade temperature in between trials. For this test, the flowers were cut at the stem and placed inside a vase with water in the sunlight.

We also observed 3 focal *B. perenis* and 4 focal *Z. aethiopica* flowers between 0900 and

1200 on 29 January in unshaded patches roughly 100 m apart. For each of the focal flowers we recorded the number of Chrysomelid visitors and the duration of each beetle's visit to the flower, from the moment when it landed on the flower until it flew off again. During this same observation period, we also performed intermittent scans every 10 minutes to record the total number of beetles on all the lily flowers in the patch (37 flowers). We divided the number of beetles by the number of flowers in the patch to get a measure of beetle population density. We used a Raytex thermal infrared thermal gun to take the temperature of a small piece of black foam, a "blackbody," resting in the flower of a *Z. aethiopica* every 5 minutes. Finally, we captured 4 flying Chrysomelids on 30 January and quickly (within 5 seconds) inserted a BAT 12 thermocouple needle to determine their internal thoracic temperature during flight.

Data analysis

We tested for patterns in Chrysomelid density and visit duration as a function of time of day with linear regressions. We compared the difference between operative temperature inside and outside of both species of flower using a paired *t*-test. To compare the thermal equilibria of Chrysomelids in each flower species, we fit Michaelis-Menten models to the temperature change over time data for each replicate time series of beetle temperatures. The Michaelis-Menten model is a standard saturation function that takes the form of equation 1, where the parameter θ_1 represents the asymptote and θ_2 represents the rate of increase in temperature over time.

$$y = \frac{\theta_1 * x}{\theta_2 + x} \quad (\text{Equation 1})$$

We compared θ_1 and θ_2 between flower treatments with two-sample *t*-tests of the means. We compared the flower species from the perspective of a beetle that is exploiting the thermal reward by estimating the duration of time over which a beetle warms when within the flower by calculating from equation 1 the time required to warm to a temperature that was 95% of the thermal asymptote for that flower type (Equation 2):

$$x = \frac{\theta_2 * y}{\theta_1 - y} \quad (\text{Equation 2})$$

We used 95% as an approximation of the point at which further thermal gains would be ecologically inconsequential.

RESULTS

For *Zantedeschia aethiopica*, Chrysomelid beetle density decreased over morning from about 0.5 to 0.3 beetles per flower (Figure 1), while the duration of visits increased from about 80 to 300 seconds over the morning ($r^2=0.15$, $P=0.002$; Figure 2).

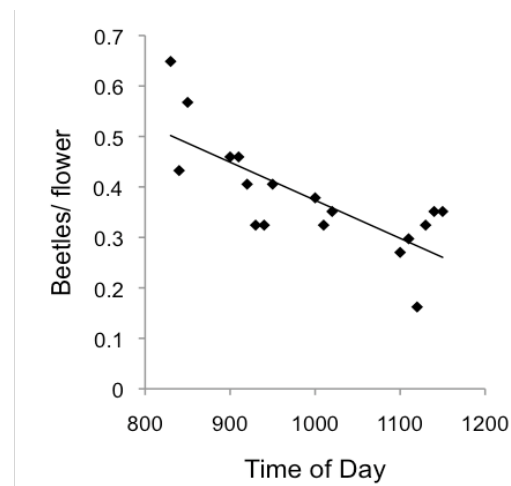


Fig 1. The average instantaneous densities of Chrysomelid beetles per 37 flowers in a patch of *Zantedeschia aethiopica* decreased over the course of a morning at Cuerici Biological Station, Costa Rica ($r^2=0.57$, $P<0.001$).

The temperature of shaded air in the area peaked at about 18° C at about 10:00 during the days we did our tests. The temperature of the black foam inside a *Z. aethiopica* flower ranged from 22.1 to 43.8° C. The internal thoracic temperatures of four beetles that were flying seconds before the measurement were: 26.3° C, 30.6° C, 30.4° C, and 27.4° C.

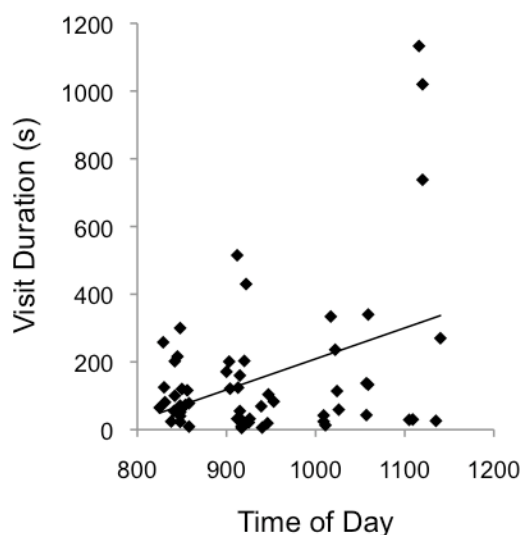


Fig 2. The duration of beetle visits to a *Zantedeschia aethiopica* patch over the course of a morning at Cuericí Biological Station, Costa Rica ($r^2 = 0.15$, $P = 0.002$). Each point represents a beetle visit to one of 4 observed flowers

Over the course of an afternoon with periods of both sun and cloud, the operative temperature of beetles was higher inside both types of flower than they were outside, with a mean difference of $2.4 \pm 0.2^\circ \text{C}$ in *Z. aethiopica* and $2.9 \pm 0.3^\circ \text{C}$ in *Bellis perennis* ($\pm 1 \text{ SE}$).

Flower type had a marked effect on the warming of beetles within the corolla (Figure 3). Beetles in *Z. aethiopica* reached an eventual equilibrium temperature of 31.8°C, which was significantly higher than those in *B. perennis*, which achieved an equilibrium temperature of 25.2°C ($t_6 = 2.60$, $P = 0.041$). Beetles placed in *Z. aethiopica* increased their temperature at a rate greater than in *B. perennis* but this differ-

ence was not significant. We observed beetles in *Z. aethiopica* to have a greater equilibrium temperature and higher rate of increase than those in the ambient air, but beetles in *B. perennis* only exhibited a rate of increase that was greater than ambient.

The estimated time for Chrysomelids to reach 95% of thermal equilibrium was 25 seconds in *B. perennis*, and 66 seconds in *Z. aethiopica*. These were a close match to the observed median duration of visits in the two species: 25 and 59 seconds, respectively (Figure 4).

DISCUSSION

Beetles in both calla lilies and daisies were warmer inside the flower than out of it. The black body temperature inside a flower was found to reach such temperatures as high as 43.8° C, much higher than the maximum shaded air temperature in the area (18° C). These results indicate that the flowers warm not only themselves, but also their insect visitors.

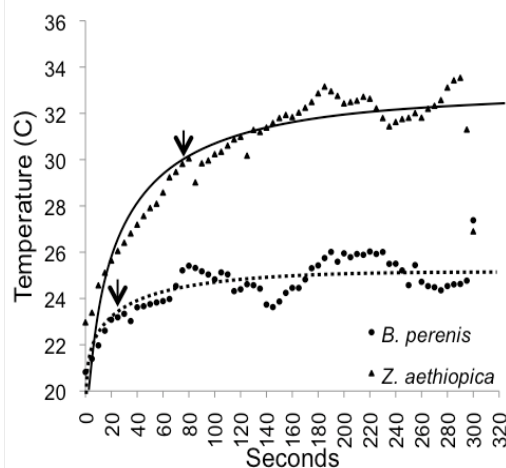


Fig 3. Operative temperature change over time in flowers of *B. perennis* and *Z. aethiopica*. The best fit Michaelis-Menten saturation model of operative temperature over time had an asymptote parameter (θ_1) of 25.11 in *B. perennis* and 31.78 in *Z. aethiopica*, and a rate parameter (θ_2) of 1.31 in *B. perennis*, and 3.47 in *Z. aethiopica*. Arrows represent the point of 95% saturation in each respective model.

Furthermore, we found that the *Z. aethiopica* and *B. perenis* warmed beetles to different maximum temperatures. The calla lily heated the beetles longer and to a higher temperature, which could be explained by the more enclosed corolla of the calla lily acting as a parabolic reflector of the sun's radiation (Dibiccari et al., 2010). In addition, this shape probably limits the heat lost to convection.

The duration of beetle's visit corresponded to the time our temperature model predicted it would stay to approach thermal equilibrium. This suggests that temperature plays a role in the visitation time of the beetle. This supports the hypothesis that beetles derive a benefit from the thermal effect of the flower. However, we do not yet know if these

proach their upper thermal tolerance. However, we believe the former explanation to be more likely, as the beetles left the two flower types after reaching different temperatures contrary to the expectation if they are avoiding physiologically dangerous temperatures. Additionally, the beetle stayed longer at the calla lilies, which would not be expected if they were leaving to avoid overheating. A thermal performance curve for this beetle, which remains unknown could differentiate between these hypotheses more conclusively. It would also be informative to manipulate flower morphology in a way that changes its thermal properties for visiting beetles.

In such extreme thermal environments as tropical montane forests we gain knowledge about how life persists and how mutualistic relationships can be impacted by temperature and the thermal physiology of individuals involved.

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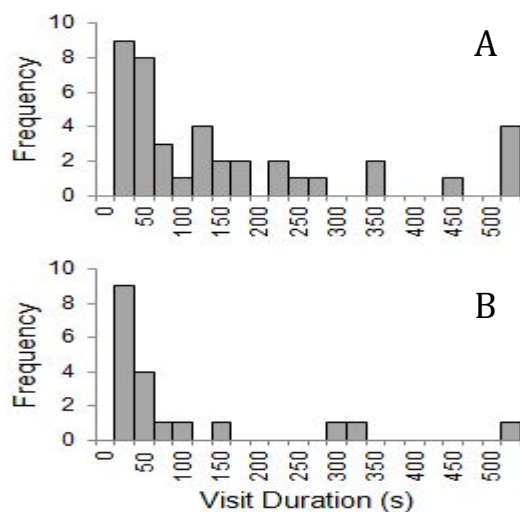


Fig 4. Frequency distributions of duration of beetle visits to *Bellis perenis* (A) and *Zantedeschia aethiopica* (B) at Cuerici Biological Station, Costa Rica. Median visit durations were 54 and 24 seconds, which corresponded to differences between flower species in the duration of warming for a visiting beetle (Fig. 3).

observed visitation times are a result of thermal equilibrium being beneficial for the visiting beetle. It could also be the case that the visiting beetles are not deriving a benefit from the flower's warming effects, but are instead being driven out by the warming as they ap-

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APPENDIX

To assist future studies, we provide here a description and image of our insect pollinator (Figure 5). We believe this beetle is in the family Chrysomelidae and the subfamily Hispanae. At a length of approximately 10 mm, this beetle was characterized by red and black coloration. Distinctive features include tarsi with four indented segments before the claw, a small tubular snout, and a ridged elytra. It also had a segmented, fairly straight, and non-oval body shape with non-club-shaped antennae.

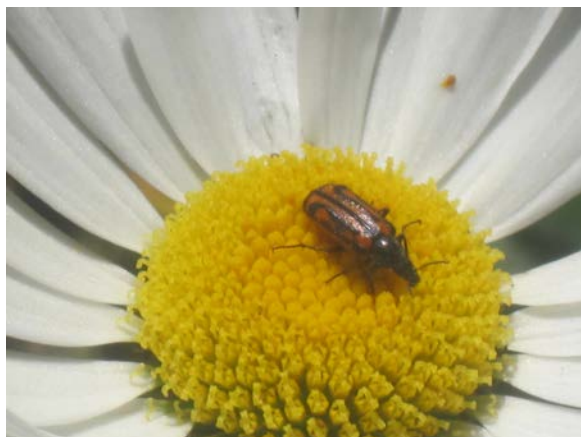


Figure 5. Chrysomelidae, Hispanae. Photo taken at Cuericí Biological Station, Costa Rica.

ON THE NATURE OF THE INTERACTIONS BETWEEN *NASUTITERMES EPHRATAE* AND *ECITON BURCHELLI*

WALES A. CARTER, MILO S. JOHNSON, AND BENJAMIN J. KESSLER

Faculty Editor: Matthew P. Ayres

Abstract: The fierce struggle between predator and prey, so commonly witnessed in nature, has existed for hundreds of millions of years. Prey species have persisted when they have evolved mechanisms for defending against or avoiding predators. In the Osa Peninsula in Costa Rica, the army ant *Eciton burchelli* is an abundant generalist predator that is broadly devastating to other invertebrates and even small predators. The termite *Nasutitermes ephratae*, another abundant social insect species in the region, appears to be a potential prey item for *E. burchelli*. We examined the current and evolutionary relationship between these two species using a series of tests designed to understand the interactions between *N. ephratae* and *E. burchelli* as compared to their interactions with other species. Our results suggest that *N. ephratae* has a species-specific defense against *E. burchelli* and that *E. burchelli* has a species-specific avoidance behavior towards *N. ephratae*. We believe these species have coevolved in an arms race and that currently *N. ephratae* has the upper hand, in accordance with the life-dinner principle.

Key words: Army ants, coevolution, life-dinner principle, arms race, termite defenses, *Nasutitermes ephratae*, *Eciton burchelli*

INTRODUCTION

Sometime after life first arose from the primordial soup, but before the explosion of modern animal life in the Cambrian, organisms began to subdue and consume other organisms to gain the energy necessary for life (Erwin *et al.* 2011). Since then, the phenomenon of predator prey interactions has become one of the most ubiquitous and important relationships in the natural world (Denno and Lewis 2009). Rigorous mathematical models have described how these predator-prey associations can be stable over geological time via negative feedbacks governing their abundances (Berryman 1992). However, our best understanding is of specialist predators that rely on one or a few sources of prey (Hassell and May 1986). The dynamics become more complicated with generalist predators (Hassell and May 1986), and the stable coexistence of prey amidst predators is more challenging to explain. For example, the history of invasive predators shows us that generalists can

continue to flourish after they have caused a severe reduction, or even extinction, of one of their prey items (Wiles *et. al.* 2003, Medina *et. al.* 2011).

On the floor of the lowland forest of the Osa Peninsula in Costa Rica, there is no generalist predator more devastating and awe-inspiring than the army ant, *Eciton burchelli*. As dawn passes and light filters through the canopy, colonies of up to 700,000 *E. burchelli* march forth from their bivouacs beneath dead logs or within the buttresses of trees (Hölldobler and Wilson 1990). By midmorning, orderly lines lead outward from the queen's abode and eventually branch into a swarm of conspicuous foragers that scour the forest floor and the trees above. The swarm, accompanied always by the shrieking cries of opportunistic antbirds, leaves a path of mortality in its wake that few invertebrates escape (Schneirla 1956). These deadly colonies, which have been compared to packs of miniature wolves on the hunt (Rettenmeyer 1983), prompted us to ask: how do other abundant insect species in the lowland jungle survive

amidst this scourge of predation? Since most antipredator strategies against army ants rely heavily upon escape as a strategy (Gotwald 1995), this question is especially pertinent for other colonial eusocial insects that live in large stationary nests, most notably other ants and termites.

Ants and termites, the “superpowers of the insect world,” have been locked in a co-evolutionary arms race for millions of years (Hölldobler and Wilson 1990, Wheeler 1936). The aggressive attacks of ants have exerted tremendous evolutionary pressures on termite species, resulting in a myriad of defensive strategies, from the enlarged snapping mandibles of *Capritermes* to the cylindrical heads that *Cryptotermes* use to plug openings in their nest (Hölldobler and Wilson 1990). The soldiers of *Nasutitermes* use a specialized “spray gun,” or rostrum, on the front of their heads to spray sticky and odorous chemicals at their attackers, with surprising accuracy for creatures that are blind (Eisner *et. al.* 1976, Hölldobler and Wilson 1990). The nature and specificity of such defenses provide clues to the history of the coevolutionary arms races between ant and termite species.

Based on initial observations that *E. burchelli* avoids the termite *Nasutitermes ephratae*, we narrowed our initial question to concern how *N. ephratae* has persisted in the presence of *E. burchelli*. To better understand the current and evolutionary nature of their relationship, we investigated: 1) if the avoidance behavior observed in *E. burchelli* is specific to *N. ephratae*; 2) if other ant species, one sister species and one distantly related species, show the same avoidance behavior; and 3) if the termites have a species-specific defense against *E. burchelli*.

METHODS

We initially observed a column of *E. burchelli* passing over a *N. ephratae* nest without attacking on 4 February 2012 in Corcovado National Park, Costa Rica. Surprised by the lack of attack by *E. burchelli*, we explored this perceived avoidance further over the next 3 days. We placed pieces of *N. ephratae* nest (with *N. ephratae* present) approximately 10 cm³ in size in an intermediate foraging column of *E. burchelli* and then observed and filmed the response of that column. We repeated this three times on columns of varying size and distance from the bivouac. We conducted this same set of three trials, filming when possible, on foraging columns of both *E. hamatum*, a congeneric army ant species characterized by column foraging and specialization in insect larvae, and *Atta colombica*, a dominant leafcutter ant species in this region (Hölldobler and Wilson 1990). These tests were designed to compare the responses of a closely related species (*E. hamatum*) and those of a distantly related ant species (*A. colombica*) to *E. burchelli*.

We also dragged a cotton swab, onto which we had previously applied the fluids of crushed termites, across the substrate of three *E. burchelli* columns to test for the possibility of a chemical signal of termite presence. With the same logic as before, we repeated this termite swab test on both *E. hamatum* and *A. colombica*, and we ran control tests using swabs from other non-ant arthropods and clean swabs. Finally, we brought individuals of our four focal species back to the lab and examined the interactions between each ant species and *N. ephratae* by placing a single ant in a Petri dish with ten termites, with three replicates for each species.

RESULTS

Each time we exposed *E. burchelli* to termites, we observed either a complete relocation or abandonment of the foraging column; smaller columns were abandoned, while larger columns were redirected. We saw this behavior both when the foraging column was on a termite nest and when it was at a location that was originally termite-free. To control for the physical disturbance of adding termite nest pieces, we placed leaves in an *E. burchelli* column. The ants were wholly undeterred and went over or through the debris in every case. When we placed several *A. colombica* individuals in the foraging column, *E. burchelli* continued without any sign of perturbation, and the leafcutters avoided the column. When we placed nonsocial arthropods, including an arachnid and an orthopteran, in the army ant column, they were immediately attacked, subdued, and dismembered by the instantly forming aggregations of *E. burchelli*. We saw a variable response to the addition of *N. ephratae* to *E. hamatum*, but responses were never greater than minor relocations and more often were nonexistent. *A. colombica* did not react or relocate its foraging column in response to the addition of termites.

We observed an immediate and dramatic avoidance reaction in *E. burchelli* when exposed to swabs of crushed termites. This was similar to their responses to termite nest pieces, but contrasted strongly with our observations during control tests. We observed no response with a clean swab, stereotypical hunting behavior and continuation of the column with an arthropod swab, and briefly increased general activity with a swab of crushed *E. burchelli*.

Atta colombica individuals did not respond to any of the swabs except for the swab infused with members of their own species,

which made the ants scatter and then resume their path. *Eciton hamatum* responded to the three *N. ephratae* swab tests variably, once ignoring the swab and twice slightly changing course at the site of the swab as if briefly following the scent. *Eciton hamatum* responded to a spider swab with slight aggregation and apparent interest, and an *E. hamatum* swab caused them to scatter but then return to their original path.

Throughout these trials, we observed a curious phenomenon when *E. burchelli* was exposed to *N. ephratae*. Occasionally an *N. ephratae* individual, in response to an approaching *E. burchelli* individual, would point its nostrum up toward the ant and, by some unknown mechanism, promote a complete and sudden paralysis in the ant, often with the stereotypical characteristic of one raised and one lowered antenna. This type of interaction was consistently present in a small number of ant individuals in each trial. We also observed this in one instance where we only presented *E. burchelli* with a cotton swab of crushed *N. ephratae*. When we touched a single ant and termite to one another in the laboratory, each held by forceps, this paralytic response seemed to be most common when the termite's rostrum came into contact with the antennae of the army ant. We witnessed no such response in either *E. hamatum* or *A. colombica* in any trial either in the field or in the lab.

The results of our ant-termite pairings in the lab were consistent across replicates within each species. *Eciton burchelli* consistently appeared to become incapacitated with reasonable rapidity, by both a combination of glued limbs and the paralysis we had previously observed. On the other hand, *E. hamatum* was incapacitated slowly by *N. ephratae* soldiers, who glued themselves to one another and to the ant, causing it to

writhe and struggle. *Atta colombica* and *N. ephratae* mostly ignored each other in this test, but we occasionally witnessed *N. ephratae* attacking *A. colombica*, presumably with glue, and *A. colombica* grooming itself in response and then continuing unhindered. (See supplementary materials for videos of our experiments).

DISCUSSION

Though termites are generally considered one of the most important food sources for army ants (Gotwald 1995), *E. burchelli* clearly avoided introduced *N. ephratae*, a behavior that was not observed when *E. burchelli* was confronted by other arthropods (*A. colombica*, an arachnid, and an orthopteran). We believe that this avoidance represents an adaptive behavior in *E. burchelli* because there would otherwise be a considerable cost to the army ant colony each time they encountered the ubiquitous *N. ephratae*. We observed this distinct avoidance behavior in response to both live termites and a swab with crushed termites, indicating that *E. burchelli* uses a chemical signal to determine the presence of termites and trigger this response. A closely related army ant species, *E. hamatum*, responded to *N. ephratae* much less consistently and stereotypically than *E. burchelli*, while *A. colombica*, a distantly related species, exhibited little or no response. Considering these results, we believe that the observed avoidance represents a newly derived synapomorphy in *E. burchelli*.

Termite soldiers have been called “a cast of nightmarish monsters,” and though *Nasutitermes ephratae* soldiers can seem innocuous enough to a casual observer, they by all means fit this description (Maeterlinck 1939). The use of monoterpene and diterpene glues by *Nasutitermis* species is well documented

(Howse 1984); we interpret the response of *N. ephratae* to *E. hamatum* that we observed in the lab as an example of their generalized response to nest invaders. In contrast, when *N. ephratae* encountered *E. burchelli*, they attacked and always elicited a temporary state of stereotyped “paralysis” in at least several ants, in which the ants stood perfectly still instead of struggling against the physical effects of glue. Termite defensive glues have been observed to contain chemicals with irritant or toxic properties (Hölldobler and Wilson 1990), so the paralysis reaction in *E. burchelli* could be the pathological effect of a selective toxin specific to them in *N. ephratae* glue. Such a toxin could have evolved as part of the defensive repertoire of *N. ephratae* against predation by *E. burchelli*. Another possibility is that paralysis is an adaptation of *E. burchelli* to specifically avoid costly contact with *N. ephratae*. The former mechanism is more parsimonious, but we do not reject the latter possibility. Further studies are necessary to distinguish between these possibilities.

In any case, our studies indicate species specificity on both of sides of the interaction between *E. burchelli* and *N. ephratae*, which implies a highly derived form of predator-prey interaction. The paralyzing attack of *N. ephratae* appears to be specifically oriented towards *E. burchelli*, and the marked avoidance behavior of *N. ephratae* seems to have only evolved in *E. burchelli*. Based on these characteristics, we believe that this interaction may be the result of coevolution. The lack of such a paralytic effect on the congeneric *E. hamatum* supports the notion that this defense has evolved to specifically target *E. burchelli*. However, it remains possible that this is a more generalized defense against attackers and that *E. hamatum*, a specialist predator on

social insects, has acquired an additional adaptation to overcome the effects of the toxin.

A particularly fascinating implication of our findings is that the adaptive response by *E. burchelli* to *N. ephratae* is one of avoidance rather than a mechanism to overcome the termite's defenses. Though these two species may not today be predator and prey, our study appears to reveal in their interactions the ghost of predation past. The termites have managed to reach a ceasefire with *E. burchelli*. While the best understood predator-prey systems involve specialist predators, which must outdo their prey or face certain death (Berryman 1992), this dichotomy does not hold for *E. burchelli*. *Eciton burchelli*, a generalist, has evolved to avoid *N. ephratae* rather than to escalate the evolutionary arms race. This appears to conform to the life-dinner principle (Dawkins and Krebs 1979), which argues that strong selection to survive, in this case on *N. ephratae* exposed to *E. Burchelli*, will trump the relatively weak reciprocal selection on a generalist predator, here *E. burchelli*. An army ant attack is a matter of life and death for an *N. ephratae* colony, and the species has clearly evolved defenses in response to this strong selection pressure. Consequently, the termites are costly and dangerous prey, and now *E. burchelli* has apparently evolved to change its diet rather than trying to subdue this dangerous quarry. This principle may serve to explain how potential prey species around the world have persisted amidst powerful generalist predators over evolutionary time. It reminds us that though nature be red in tooth and claw and species locked in battles that wage for millions of years, occasionally a prey species may escape the yoke of predation, leaving only whispered clues for us to follow into their violent past.

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EVIDENCE FOR A COMMENSALISM BETWEEN FISH-EATING BIRDS AND LARGE MARINE PREDATORS

ANNA L. DEFFEBACH, SHEA E. FLANAGAN, MADILYN M. GAMBLE, AMY E. VAN SCOYOC

Faculty Editor: Matthew P. Ayres

Abstract: Species interactions can contribute to biodiversity. For example, commensalisms can increase biodiversity by creating more niches in the ecosystem. We tested for evidence of a commensalism between large fish-eating birds (herons and egrets; family Ardeidae) and large marine predators in the Rio Sirena lagoon in Corcovado National Park. We observed bird feeding behaviors in the Rio Sirena lagoon (where large marine predators are present at high tide) and in the Rio Claro lagoon (where large marine predators are rare or absent). In the Rio Sirena lagoon, bird feeding activity peaked at high tide following a sinusoidal function that matched the tidal cycle. In contrast, Ardeid birds were rare or absent (1 or 0 birds) in the Rio Claro lagoon throughout the day, and there was no trend in feeding behavior with changing tide. Furthermore, the numerous snowy egrets (*Egretta thula*) in the Rio Sirena lagoon frequently employed a passive hunting tactic that depended on small fish jumping onto the shore, apparently fleeing large predators. A commensalism between birds and large marine predators seems to contribute to the biodiversity of the Rio Sirena ecosystem.

Key Words: commensalism, Ardeidae, biodiversity, tidal cycles, *Egretta thula*, fish-eating bats

INTRODUCTION

Beneficial associations among species (i.e., mutualisms and commensalisms) can add to species diversity by creating niches in the community that would not be possible without the biotic interactions. In this sense, mutualisms and commensalisms differ somewhat from predation interactions, which can either enhance or depress species diversity. For example, the introduction of non-native predators has repeatedly led to extinctions of indigenous species, especially in the last century, even though in other situations predation can permit the coexistence of more species than would otherwise be possible. Such is the case of the starfish *Pisaster ochraceus*, which maintains barnacle diversity in marine ecosystems by preying preferentially on the dominant species of barnacle, thus providing competitive release for other species (Holt 2009). In the tropics, the already high number of species enriches the opportunities for net-

works of species interactions to develop (Willig et al. 2003).

Though the tropics exhibit characteristically high biodiversity, it is not homogeneously distributed (Pianka 1966). By examining habitats at a higher resolution, it becomes evident that the spatial clustering of species interactions within a tropical habitat may open niches not present in similar environments nearby. Hence, intense intermingling of organisms in the tropical environment forms radiating “trophic oases,” where combinations of interspecific interactions can provide specific, small-scale ecological opportunities that would not otherwise exist (Schemske et al. 2009).

Our study investigated the potential causes for the observed difference in bird diversity between two geomorphologically similar lagoons in Corcovado National Park, Costa Rica. The Rio Sirena lagoon is home to many species of birds and fish, as well as large marine predators that frequent the lagoon during high tide. Though the Rio Claro lagoon

hosts similar populations of small fish, it lacks the influx of large marine predators that occurs in the Rio Sirena at high tide. Initial observations suggested that marine predators' feeding behavior push schools of smaller fish close to the shore where they are more accessible to large fish-eating birds of the family Ardeidae (especially herons and egrets). Previous studies have indicated a commensalism at night between fishing bats (*Noctilio leporinus*) and what appeared to be the same or similar community of marine predators. In this interaction, the bat hunting tactics seem to take advantage of the activity of large marine predators at high tide (Yale et al. 1998, Veysey et al. 2000, Leslie et al. 2001, Spinoso et al. 2008). These investigators hypothesized that the lack of fish-eating bats in the Rio Claro was at least partly because the less intense influx of marine predators at high tide fails to create such a favorable foraging environment for the bats. If there were an ecological parallel with the diurnally feeding birds, one would expect a corresponding difference in the feeding activity and abundance of the bird communities between the two lagoons during high tide.

METHODS

Study Sites

The Rio Sirena and Rio Claro lagoons were similar in width and length. Our study sites were approximately 50 m across, beginning at the narrowest point of the mouth of the lagoons and extending 75 m toward the river. The lagoons are located approximately 1500 m apart and both empty into the Pacific Ocean. Both study sites had similar sand substrate, bank vegetation, and available perching habitat for bird species (with mangroves and wide shores). There was a constant presence of similar populations of small fish that

apparently are suitable prey items for herons and egrets.

The Rio Sirena lagoon was deeper and more turbid than the Rio Claro lagoon. Additionally, Rio Sirena had steeper banks at low tide than Rio Claro, but at high tide, the slopes of both lagoons' banks were similarly gradual. The Rio Sirena lagoon maintained a population of crocodiles and large predatory fish, as well as bull sharks (Logan, M.L. *pers. comm.*) that frequented the lagoon mouth during high tides. Neither crocodiles nor bull sharks were evident in the Rio Claro lagoon.

Sampling methods

We performed a pilot study on 4 February 2012, in which we observed bird activity and small fish being chased toward the shore by larger marine predators. We observed the Rio Sirena lagoon from 09:30 to 11:30 and 12:15 to 15:30 in 15-minute continuous segments. The high tide occurred at 12:24. We counted the number of audible splashes occurring in each 15-minute observation period and recorded the number of each Ardeid species present within the study site. We hypothesized that the number of splashes would function as a proxy for lagoon predator activity. Throughout each observation period, we also scored each bird's activity on a qualitative scale of 1 to 3 defined as 1) unengaged, preening, or sleeping, 2) positioned near the water or ready to fish, or 3) actively engaged in fishing behavior. Additionally, we recorded interactions among the birds and other potentially relevant observations.

On the following day, we simultaneously observed the Rio Sirena and Rio Claro lagoons from 08:30 to 11:15 and 12:45 to 16:15, following the same methods described above. The high tide occurred at 13:16. We also paid particular note to the activity patterns and feeding behavior of snowy egrets (*Egretta thu-*

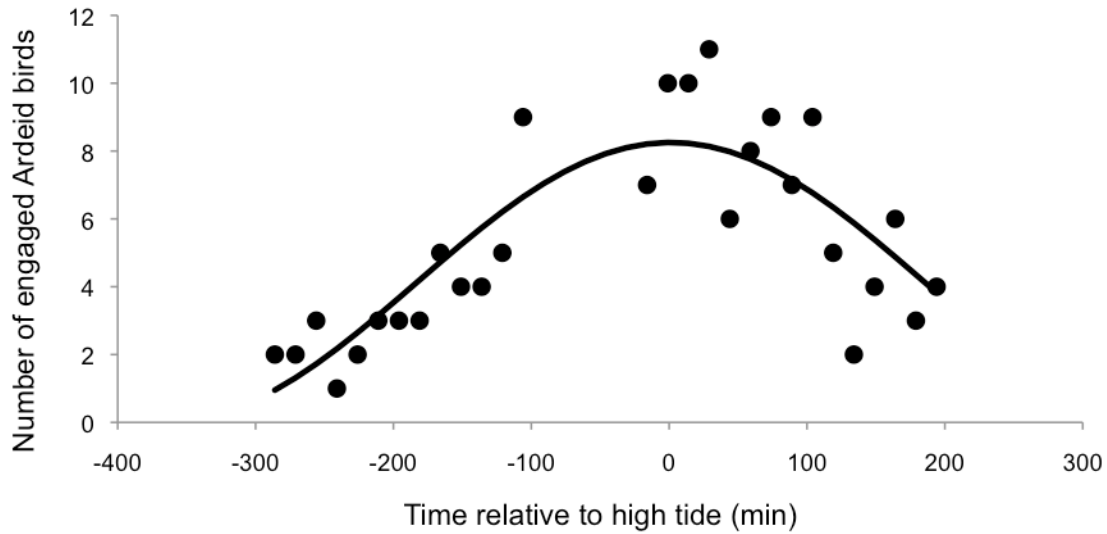


Fig. 1. Relationship between tide and the number of herons and egrets engaged in hunting in the Rio Sirena lagoon. The number of engaged birds is described as a sinusoidal function of tide, peaking 1 ± 11 minutes after high tide (mean ± 1 SE). Other parameters are reported in Table 1.

la), which were the most numerous species. We defined active feeding behavior as birds moving along the shore and thrusting their beaks into the water. We defined passive feeding behavior as birds that appeared to be waiting for fish to be chased out of the water by marine predators.

Data analysis

We fit a parabolic curve to examine the relationship between number of splashes in each lagoon over the tidal cycle. We plotted the number of engaged birds (birds with an activity score of 2 or 3) against the progression of the tidal cycle for both lagoons. We then developed a sinusoidal function to describe the relationship between number of engaged birds and tide:

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

Equation 1

The function had four parameters: p (period) was fixed at 737 minutes, which was the

tidal period on the day of experimentation. The remaining three parameters were estimated from the data: t represented the time relative to high tide when the number of engaged birds was maximized; n (nadir) represented the minimum number of engaged birds through the tidal cycle; and a (amplitude) represented the difference between the maximum and minimum numbers of engaged birds. We used maximum likelihood to estimate t , a , and n (JMP 9.0, SAS Institute, Cary, NC). Initially this produced a slightly negative estimate for n (-0.3), so we constrained n to ≥ 0 , which is the possible range for minimum bird abundance (Table 1). Finally, we analyzed how active and passive feeding events for *E. thula* related to the tidal cycle.

RESULTS

Over the course of the study, we observed 6 Ardeid species in the Rio Sirena lagoon (*Egretta caerulea*, *Egretta tricolor*, *Ardea alba*, *Egretta thula*, *Tigrisoma mexicanum*, and *Eudo-*

cimus albus). *E. thula* was by far the most numerous of these. As many as 9 individuals could be observed at a single time. The other species seemed to be represented by only 1 to 3 individuals. We observed only 2 individual Ardeid birds in the Rio Claro lagoon, one each *E. caerulea* and *E. tricolor*. *E. thula* was the only bird species in either lagoon that we observed engaging in passive feeding behaviors. Large marine predators were not observed at any time in the Rio Claro lagoon. *E. thula* engaged in passive feeding more frequently during high tide and that active feeding at this time was much less frequent. A congregation of at least 8 large bull sharks (up to 3 m long) were observed actively feeding just outside the Rio Sirena lagoon during the hour following high tide each day between 3 and 5 February 2012 (Logan, M.L. *pers. comm.*).

The number of splashes did not correlate with time in either lagoon (Rio Sirena: $P = 0.35$, Rio Claro: $P = 0.55$). There were up to 11 herons and egrets engaged in hunting at the Rio Sirena lagoon. The number of engaged birds in the Rio Sirena lagoon was very well described as a sinusoidal function in which the maximum number of birds engaged occurred at $t = 1 \pm 11$ (mean ± 1 SE) minutes after high tide. The estimated amplitude was $a = 8.2 \pm 0.4$ (mean ± 1 SE), which represents the peak abundance of engaged birds during the tidal cycle. We did not try to fit a sinusoidal function to the Rio Claro bird data because there were so few birds.

TABLE 1. Parameter estimates for Equation 1.

Parameter	Mean	Standard Error
t	1.22	10.96
p	737	N/A
a	8.25	0.44
n	0	N/A

DISCUSSION

Our results suggested a strong commensalism between Ardeid birds and large marine predators in the Rio Sirena lagoon. Since the number of engaged birds was well described by our sinusoidal model and peaked at high tide, we inferred that some change in the Rio Sirena lagoon at high tide was affecting bird feeding behavior. Because the small fish that the birds eat were observed at all tides, while the large marine predators were far more abundant at high tide than low tide, we concluded that the presence of the large marine predators was the factor affecting bird feeding behavior. This conclusion was supported by the low bird abundance and lack of any *Egretta thula* in the Rio Claro lagoon, where the small fish food source was available regardless of tide but large marine predators were absent. Had bird abundance and activity increased significantly at mid-day in the Rio Claro lagoon, it would have been difficult to eliminate the possibility that patterns in bird feeding behavior were a function of time rather than tide. Since bird activity in the Rio Claro lagoon remained low regardless of time or tide, this strengthens the inference that bird feeding behavior is a function of temporal cycles in marine predators, rather than time since dawn or tide per se.

Additional support for a commensalism came from the observed temporal shift in *E. thula* feeding strategies. The fact that passive feeding strategies peaked at high tide while active feeding drastically decreased at high tide suggested a relationship between tide and feeding behavior. According to Stiles and Skutch (1989), *E. thula* are commonly observed taking advantage of other bird feeding activity that drives small fish to the surface. Our study indicated that *E. thula* exhibit simi-

larly opportunistic feeding strategies in the presence of large marine predators.

Although we did not find a correlation between number of splashes and tide, this may have been because many predation events occur below the surface, and are especially well hidden at high tide in deeper water. Previous studies also found splash frequency to be an unreliable predictor of activity (Leslie et al. 2001). We interpreted the presence of hunting bull sharks outside the Rio Sirena lagoon just after high tide as an indication of an outflux of large marine fish (potential prey for the sharks). These larger fish were likely feeding on smaller lagoon fish during high tide, causing the smallest fish (2 - 6 cm) to jump out of the water near the shore. It was these smallest fish on which the birds were preying. Small fish jumping out of the water further supported our assumption that large marine predators were present and active during high tide.

Our results supported the hypothesis that the biodiversity of the Rio Sirena lagoon is enriched by a commensalism in which large marine predators create feeding opportunities for fish-eating birds as well as fish-eating bats. This implies that the large marine predators function as a kind of keystone species in the Rio Sirena lagoon. Future studies could test this further by observing both lagoons when high tide does not coincide with mid-day. The fact that similar levels of bird diversity were not evident in the morphologically similar Rio Claro lagoon only 1500 m away may be evidence of trophic oases and tropical habitat heterogeneity induced by species interactions in Corcovado National Park. Insight into the patchiness of species interactions and their emergent properties on a small scale contributes to understanding the processes that generate biodiversity in tropical ecosystems.

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NICHE PARTITIONING OF A BRIEFLY BOUNTIFUL FOOD RESOURCE: FRUGIVORY AT A FIG TREE

M. HEMAYAT R. CHOWDHURY, NINA B. FRANKEL, AND JESSE T. RIEB

Faculty Editor: Matthew Ayres

Abstract: Niche partitioning may allow for the coexistence of species by dividing a shared resource temporally, spatially or morphologically. In this study, we examined the extent and mechanisms by which niche partitioning may occur at an ephemeral resource, using a fruiting fig tree as a model system. For three days we observed the foraging behavior of vertebrate frugivores that visited a fruiting *Ficus insipida*, and examined fallen figs for internally feeding frugivorous insect larvae. We tested if collared peccaries had a preference for whole uneaten figs or figs that had partially been eaten by spider monkeys. We observed six different vertebrate species visiting the fig tree, with spider monkeys (*Ateles geoffroyi*) and collared peccaries (*Pecari tajacu*) being our most frequent visitors. Figs that were partially eaten by monkeys were less likely to contain internal larvae, and peccaries were more likely to eat whole figs than figs that had been partially eaten by monkeys. Our results suggest some spatial and morphological partitioning of the ephemeral fruiting fig resource between monkeys, peccaries, and larvae. Modest temporal patterns of feeding visits by vertebrates seemed to be more attributable to daily activity patterns and/or the effect of temperature than to a temporal partitioning of the fig resource.

Key words: ephemeral resource, *Ficus insipida*, niche partitioning, *Ateles geoffroyi*, *Pecari tajacu*

INTRODUCTION

Niche partitioning can increase biodiversity within a community by increasing the probability of coexistence of pairs of species. Two species that are ecologically equivalent and use the same limited resource cannot readily coexist because interspecific competition tends to result in the competitive exclusion of one of the species. Dividing the use of the shared environment into different functional niches increases the probability of coexistence between two species by decreasing the likelihood of strong interspecific competition. Niche partitioning can be spatial, temporal, or morphological. Spatial partitioning of resources occurs when organisms use the same resource but in different places. Temporal partitioning of resources can include dividing consumption of food resources based on time of day or time of year. Morphological partitioning occurs when organisms differ in phys-

ical characteristics, allowing each to be better suited to exploit a slightly different resource. These mechanisms of niche partitioning decrease the likelihood of competitive exclusion, and can thus increase species richness within a community.

While niche partitioning can be explained in long-lasting communities by the evolution of distinct functional niches, it is less clear if niche partitioning occurs on short time scales. Some resources are highly ephemeral, lasting for much less time than one generation of the organisms that utilize it (Shorrocks *et al.* 1981). One example of an ephemeral resource is a fruiting fig tree. Fig trees fruit synchronously within a tree but are temporally asynchronous within a population. Due to the large amount of fruit present, a species-rich assemblage of frugivores is attracted to a fruiting fig tree (Janzen 1979). In this study we sought to understand the extent and nature of niche partitioning by foragers at the ephemeral resource that is a fruiting fig

tree.

METHODS

We observed a fruiting fig tree, *Ficus insipida*, in Corcovado National Park, Costa Rica from 4-6 February 2012. Our focal tree was in an early stage of fruiting, as determined by the high abundance of fruits on the tree, and the low abundance of fruits on the ground. We conducted observations between 0750-1120 and 1220-1730 on day 1, between 0530-0628, 0710-1120, and 1400-1732 on day 2, and between 0530-0625, 0707-1130, 1220-1320, and 1616-1735 on day 3. All three observers were present during peak activity times (0730-1030 and 1630-1730) and at least one observer was present during all other observation times. We positioned ourselves 4-6 m from the *F. insipida* and silently observed visiting vertebrates through binoculars. We noted the time of arrival and departure of all vertebrate species that we observed foraging on figs in or directly below our fig tree. We also measured shaded air temperature near our fig tree using a HOBO TidbiT v2 temperature logger (On-

set, Bourne, MA).

To assess spider monkey feeding preferences, we collected 10 partially eaten (by spider monkeys) and 10 uneaten figs that were found on the ground under our *Ficus insipida*. We also observed spider monkeys in another fig tree (of an unidentified species of the same genus) in a later stage of fruiting and collected any figs dropped by the monkeys. We cut open figs from both trees and examined them for invertebrates. We identified a fig wasp parasitoid (Hymenoptera: Torymidae; Gu 2008) as well as five other morphotypes of insect larvae (hereafter collectively referred to as larvae) inside the figs we collected.

To test if peccaries had a preference for whole uneaten figs or figs that had been partially eaten by monkeys, we haphazardly set up five 1 × 1 m plots within an 8 m radius of our fig tree. We placed five partially eaten and five whole, uneaten figs in each of these plots and waited for a peccary herd to visit. After we had observed peccaries foraging in our plots, we noted the number of each type of fig they had eaten.

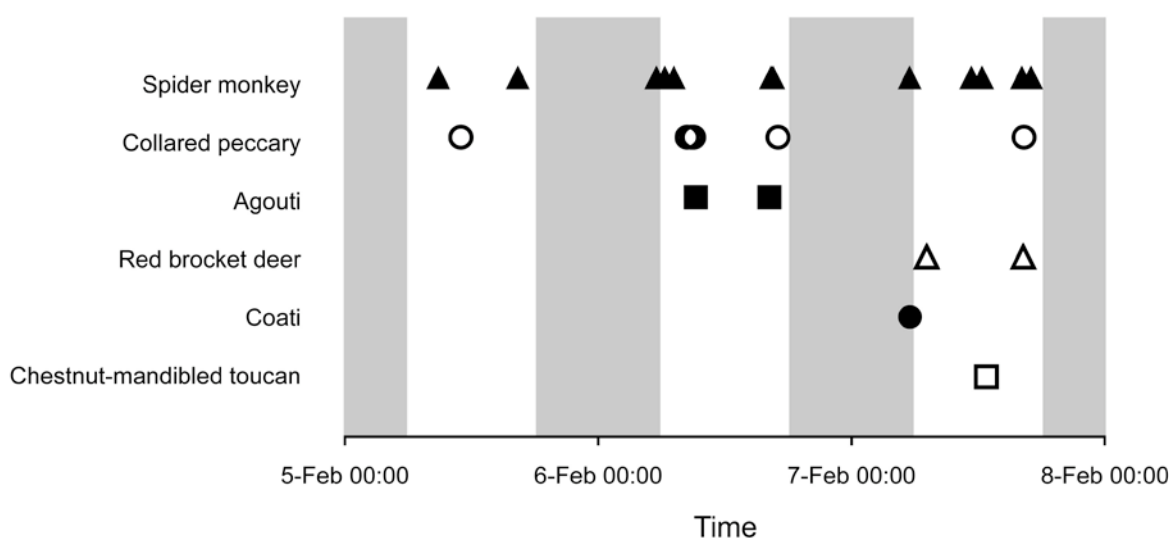


FIGURE 1: Observed visits to a fruiting *Ficus insipida* by vertebrate frugivores over a three day period. Points indicate the beginning of a visit. White and gray bars indicate daytime and nighttime respectively. We only observed the tree during the day.

Statistical Analyses

We used a contingency analysis with Pearson's χ^2 test to determine whether figs that had or had not been partially eaten by spider monkeys were more likely to contain larvae. We used a paired t-test to compare peccary consumption of uneaten or partially eaten figs. All statistical analyses were performed using JMP 9 software (SAS Institute, Cary, NC).

RESULTS

We observed Geoffroy's spider monkeys (*Ateles geoffroyi*), collared peccaries (*Pecari tajacu*), agoutis (*Dasyprocta punctata*), red brocket deer (*Mazama americana*), a white-nosed coati (*Nasua narica*) and a chestnut-mandibled toucan (*Ramphastos ambiguus swainsonii*) feeding on figs in or directly under our fig tree (*Ficus insipida*). Geoffroy's spider monkeys and collared peccaries were the most frequent visitors. Troops of spider monkeys and herds of collared peccaries visited the site and fed on figs ten and five times re-

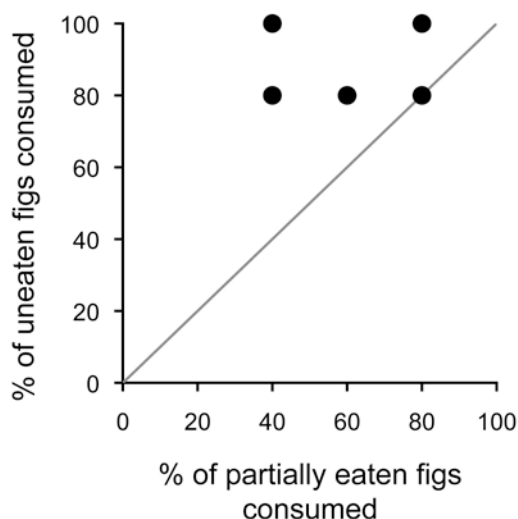


FIG. 3: Collared peccaries were more likely to consume whole figs than those that were partially eaten (paired- $t_4 = 2.75$, $P = 0.052$). Each point represents one test plot. The gray line indicates equal consumption of partially eaten and uneaten figs.

spectively, and were observed at the tree at least once each day. The number of species visiting the tree increased each day: 2 on day 1, 3 on day 2, and 5 on day 3 (Fig. 1).

Invertebrate larvae were significantly more likely to be inside of whole uneaten figs than figs partially eaten by spider monkeys (Pearson's $\chi^2 = 7.94$, $P = 0.0048$, $n = 32$; Fig. 2). Our plots showed that collared peccaries were significantly more likely to eat whole uneaten figs than ones partially eaten by spi-

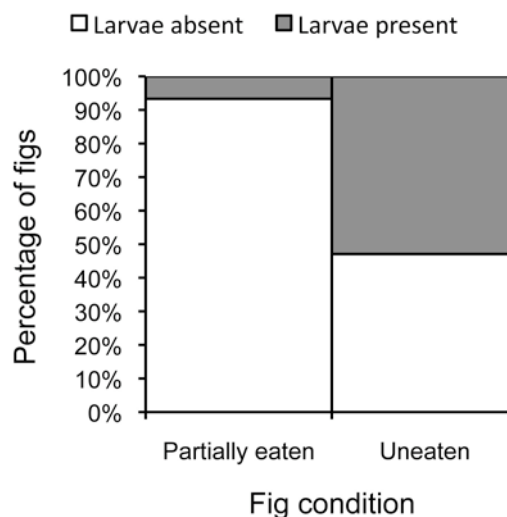


FIG. 2: Partially eaten figs were less likely to contain insect larvae than uneaten figs (Pearson's $\chi^2 = 7.94$, $P = 0.005$, $n = 32$).

der monkeys ($t_4 = -2.75$, $P = 0.052$; Fig. 3). Peccaries consumed 74 ± 11 percent of the figs in the plots (mean ± 1 standard deviation).

DISCUSSION

Our results suggested some temporal patterns in vertebrate visits to our fruiting *Ficus insipida* over our three day study period. Based on troop behavior and the number and size of juveniles, we believe that the same troops of collared peccaries and spider monkeys visited the tree each day. This implies that the troops were aware that the fruit on the tree was ripening, and visited it each day

to check for the presence of edible figs. Over the three days of observation, spider monkey groups became larger. Since spider monkeys are known to exhibit fission-fusion foraging behavior (Symington 1990), this likely means that the spider monkeys were adjusting their foraging group size in response to the increasing abundance of ripe figs. We also observed more species visiting the tree each day, suggesting that more species were discovering the tree as the fruits became riper. During each day, we observed a greater number of visits in the morning and the evening, with few visits during the middle of the day between 1200 and 1600. This time period corresponded with the hottest part of the day, as temperature peaked each day at approximately 1400. This pattern could indicate temporal partitioning in fig tree use, but it seems more likely that it just reflects patterns of animal activity related to time and/or temperature.

Although we did not find clear temporal partitioning, we did find evidence of other forms of niche partitioning, especially between the three most abundant species of fig consumers in our observations: spider monkeys, collared peccaries, and larvae. These three taxa occupy different spatial niches, with the monkeys foraging in the canopy, peccaries foraging on the ground, and the larvae feeding inside the fruit. At least early in the fruiting period, a few figs fall to the ground on their own, but the majority of figs that fall are knocked down by monkeys and other canopy visitors. This suggests that foraging by monkeys directly benefits the peccaries by increasing the availability of fruit on the ground. It could have been chance with only three days of observation, but we noted that peccaries always visited the tree within three hours after a troop of monkeys had passed through.

We also found evidence of morphological

partitioning in the feeding preferences of the different species. Spider monkeys prefer fruits that do not contain larvae. This suggests that the monkeys are able to determine whether a fig is infested prior to biting into it, whether by sight, smell, feel, or some other mechanism. On the other hand, peccaries were more likely to eat whole fruits that had not been bitten by monkeys, which presumably are more likely to have larvae in them. This may be just because foraging for whole figs is more efficient; whole figs provide more food per bite, and, when the inside of the fruit is facing up, half-eaten fruits can blend in with the forest floor. Alternatively, the larvae may provide a nutritional benefit to the peccaries in the form of protein. The larvae themselves may be actively involved in these feeding preferences. Though consumption of larvae may not actually be detrimental to spider monkeys, the larvae rely on fruits remaining uneaten long enough for their development into adults, and thus are under selective pressure to make the figs as undesirable as possible to potential frugivores (Janzen 1977). As voracious generalists, peccaries may have evolved to tolerate the effects of the larvae, and thus do not display the same aversion to infested fruits as do the monkeys.

Compared to a previous study of a fruiting fig in Corcovado National Park (Alexander *et al.* 2002), we observed a lower diversity of ground-foraging vertebrates. Their study observed crested guans, little tinamous, and white-tipped doves foraging at their tree, as well as much more frequent and continuous visits by agoutis and coatis. We think that our tree was in an earlier stage of fruiting than that observed in 2002, which had dropped the majority of its fruits by the third day of observation. The fruiting stage could have had an effect on the community of foragers. Another difference between our studies that

could have affected the community of foragers was the abundance of peccaries. Over the last ten years, protected lowland rainforests across Costa Rica have experienced alarming increases in peccary populations (Deffebach et al. 2012) The 2002 study did not record any peccary visits, while peccaries were one of the most frequent and abundant visitors to our tree. In our experimental manipulation, we found that peccaries consumed 74% of the figs available in our plots. This implies that peccaries may be having a significant impact on the community of ground foragers through competitive exclusion.

Our study indicates that niche partitioning exists even for a briefly bountiful resource such as a fruiting fig tree. Despite synchronous fruiting, a fig tree could potentially still be a limited resource because ripe fruits are not accessible to all frugivores at all times. If an ephemeral resource were limited, niche partitioning would diminish the probability of interspecific competition. Decreasing interspecific competition at an ephemeral resource could increase the number of species that can use the resource, just as niche partitioning can increase biodiversity in communities that have evolved over many generations.

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A COLORFUL TRADEOFF: BRIGHT PIGMENTATION AT THE COST OF IMMUNE FUNCTION IN *ASTYANAX AENEUS*

ROBIN COSTELLO, MADELINE KREHER, AND JAMIE MCLAUGHLIN

FACULTY EDITOR: MATTHEW AYRES

Abstract: Competing environmental pressures can present tradeoffs on populations' phenotypes. Bright coloration from carotenoid pigmentation is subject to pressures and limitations from variability in visibility, conspecific social signaling, disease, and limited pigment availability. We measured the effect of these pressures on anal fin color intensity in *Astyanax aeneus*. We found no correlation between visibility, conspecific social signaling, or pigment availability on color intensity. However, brighter *A. aeneus* populations exhibited more signs of disease. This suggests that allocation tradeoffs have an important effect on fitness as fish must choose between utilizing carotenoids for immune response or pigmentation.

Key Words: *Astyanax aeneus*, carotenoids, honest signaling, phenotypic plasticity, selective pressures, tradeoffs

INTRODUCTION

Environmental pressures shape the morphology of a population, promoting traits that increase fitness. Variation in the phenotype of a population frequently represents tradeoffs from competing environmental pressures. This range in phenotype may be mediated either intergenerationally by natural selection or intragenerationally by phenotypic plasticity.

Bright coloring is highly influenced by competing environmental pressures. The selection pressures and constraints that we expect to influence color generated from carotenoid pigments include visibility, conspecific social signaling, disease, and limited carotenoid availability (Table 1).

Light availability determines the visibility of color. As visibility decreases in an environment, more pigment is needed to convey the same level of color. Therefore, with less light in the environment, color is more advantageous, so optimal color will increase.

The importance of conspecific social signaling could similarly affect the tradeoff

of color phenotype. With an increase in population size, intraspecific competition for mate selection and dominance may increase. As population size increases, an individual will interact with a greater number of individuals, making conspecific signaling more important and coloration brighter. Alternatively, the increased pool of potential mates may decrease social signaling pressures.

Carotenoids, a chemical that many organisms use as a red and orange pigment, may also act as an antioxidant that enhances the cell-mediated immune response to disease (Hughes 1999; Lozano 1994). This creates an additional tradeoff between more color and enhanced immune systems. Presumably optimal color should decrease in the presence of disease as fish allocate more carotenoids to the immune system. Thus, a colorful population may fight disease less effectively.

As most organisms sequester carotenoids from the environment, resource availability may limit color production. With a finite supply of carotenoids, the production of

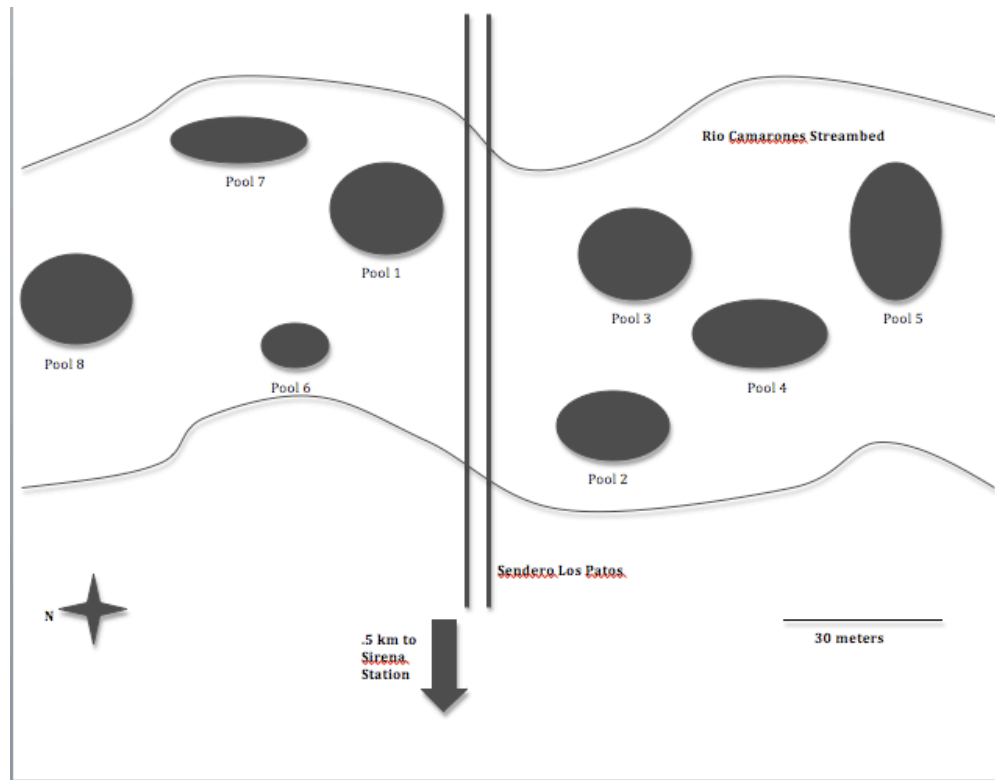


Figure 1. A graphical map of pool location and distribution in the Rio Camarones during the dry season February 2012. The pool sizes are scaled relative to each other.

color becomes more costly as the pressure on allocation tradeoffs increases. Individuals must make more frugal choices between pigmentation and immune function, so optimal color should decrease.

In general as the disadvantages of strong coloration increase and the advantages decrease, color intensity will decrease.

METHODS

To examine the environmental pressures that influence color, we studied *Astyanax aeneus* in Corcovado National Park, Costa Rica. *A. aeneus* is a small social fish with a bright orange anal fin. Similar to the scarlet macaw, *A. aeneus* do not exhibit sexual dimorphism, with both males and females displaying this orange color. During the wet season (May to Nov.), this species lives in large, continuous populations in

ivers and streams. As the water level declines in the dry season (Dec. to Apr.), populations in smaller stream become divided among small, temporally-stable, isolated pools.

We added orange-colored seeds (*Stemademia* spp.) and non-orange-colored seeds (*Ficus* spp.) to pools of *A. aeneus* in the dry riverbed of the Rio Camarones on Los Patos trail in the Sirena Biological Station. While the *Stemademia* and *Ficus* seeds are naturally very different sizes, we visually approximated the same mass of seeds and added them to the pools. We counted the number of attacks on both seed items in the first minute. We repeated the experiment 12 using different pools of *A. aeneus* as replicates. We performed a t-test to compare the attacks on the two different seeds.



Figure 2. A view from above of an outgrowth we observed on the *A. aeneus*. This outgrowth is assumed to be an indicator of poor immune function and may be either parasites or cancer.

On February 4 and 5, 2012, we temporarily removed 15-20 *A. aeneus* from each of eight pools in the dry Rio Camarones (Fig 1). We did not find *A. aeneus* in the pools of the other dried rivers near the Sirena Biological Station. We photographed each individual and quantified color intensity using Photoshop. We cropped out the orange fins of each photo, compressed the information into two pixels, and measured Saturation as a metric of color intensity. While we created a measure of color intensity de novo, we later learned that a common method in behavioral ecology is to measure “redness” is to use the equation $R/((R+G+B)/3)$ where R is red, G is green, and B is the blue index of each pixel (Frischknecht 1993; Barber et.al, 2000). We performed an ANOVA to compare color intensity among the eight pools.

To estimate the population of each pool, each researcher visually estimated the population and we recorded the average of the three observations. We performed a correlation analysis with population size and color intensity.

Many *A. aeneus* displayed an outgrowth apparently indicating some pathology (per-

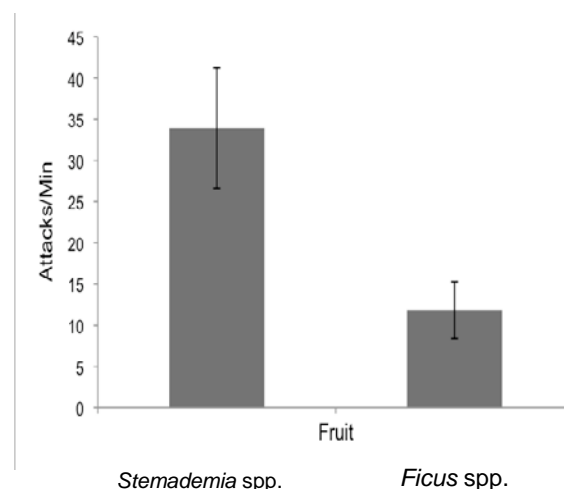


Figure 3. The number of *A. aeneus* attacks was higher on orange *Stemademia* fruit than on *Ficus* fruit of similar size. Error bars show standard errors (7.29 and 3.46 respectively).

haps parasites) likely indicating a compromised immune system (Fig 2). To test the effects of immune function on color intensity among pools, we measured the outgrowth levels of the pools. We qualitatively classified the eight pools into four categories of outgrowth level. We classified pools in category 1 when we observed 1 to 2 fish with outgrowths; in category 2 when we observed 3 to 4 fish with outgrowths; in category 3 when we observed 5 to 7 fish with outgrowths; and in category 4 when we observed 8 to 10 fish with outgrowths after 1

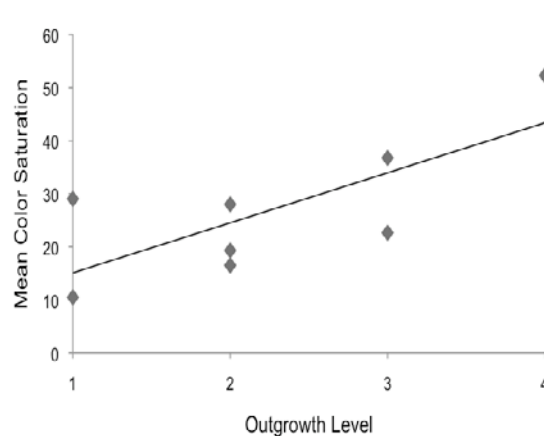


Figure 4. Pools with a higher quantity of *A. aeneus* with outgrowths contained higher average saturation of *A. aeneus* color.

minute of observation. We performed a correlation analysis between outgrowth level and color intensity.

We measured canopy cover as a proxy for light availability at each pool. We used a concave spherical densiometer to measure the amount of canopy cover in each cardinal direction at the center of every pool. We performed a correlation analysis between canopy cover and color intensity among pools.

We attempted to quantify the amount of available carotenoids at each pool by estimating the abundance of *Heliconia*, whose numerous colorful bracts represent a potential source of carotenoids. We qualitatively classified the *Heliconia* abundance at the eight pools into categories 1-3. We classified a pool in category 1 when we observed only 1 or 2 *Heliconia*; in category 2 when we observed 3-14 *Heliconia*; and in category 3 when we observed more than 15 *Heliconia*. We performed a correlation analysis between *Heliconia* level and color intensity. All statistical tests were performed in JMP 9.

RESULTS

A. aeneus significantly preferred orange *Stemmademia* seeds over *Ficus* seeds ($t_{15.7} = 2.86$, $P = 0.01$; Fig 3).

Saturation of color in the anal fin of *A. aeneus* differed within and among pools. The color saturation between individual *A. aeneus* ranged from 3 to 85 on a scale of 0-100. Furthermore, orange saturation varied among pools ($F_{7,134} = 20.83$, $P < 0.0001$).

The pool population size ranged from 55 to 567. We found no correlation between pool population and color intensity ($r = -0.14$, $p = 0.73$).

Outgrowth level and color saturation were the only correlated variables from our

correlation analysis (Table 1). Pools with a higher outgrowth level contained fish with more intense color ($r = 0.75$, $P = 0.03$; Fig 4). We found no correlation between light availability and color intensity ($r = 0.22$, $P = 0.60$). We also found no correlation between *Heliconia* density and *A. aeneus* color ($r = -0.35$, $P = 0.39$).

DISCUSSION

The spectrum of color saturation within and among pools is consistent with the hypothesis that competing environmental pressures influence *A. aeneus* color. Variation among pools could be a result of either natural selection or phenotypic plasticity. Since the fish were one population in December and live several years, the differences observed among pools seem to be intragenerational, a result of phenotypic plasticity. However, as natural selection may act rapidly, it cannot be eliminated as a possible explanation for the differing color intensities among pools.

Abundance, visibility, and resource availability did not correlate with coloration in *A. aeneus*, implying that they are not pressures in determining color. As we did not control for confounding variables, these pressures could still be operating on *A. aeneus* coloration but weakly relative to other pressures. Furthermore, we may not have fully captured all the components of these pressures. For example, resource availability could be measured more precisely by identifying sources of carotenoids other than *Heliconia*. Similarly, to fully account for the effect of visibility on coloration, substrate type and turbidity in the water should be considered along with canopy cover.

Another possible explanation for the variation in color is an allocation tradeoff between immune function and pigmentation. This allocation tradeoff is only relevant if carotenoids are limited, and *A. aeneus* must choose between health and color. The observed preference of *A. aeneus* for orange colored seeds over non-orange colored seeds of similar nutritional value suggests that *A. aeneus* cannot generate their own pigment and must acquire it from the environment around them. However, this preference may be due to some other factor, such as taste. Still, we interpret their frenzied interest in the orange seeds as a possible indicator that natural carotenoid resources are limited in their environment and therefore must be selectively allocated by an individual. This provides further evidence of phenotypic plasticity as a mechanism for the variation in color.

We assumed that the outgrowths on the fish were an indicator of disease. Though currently unidentified, the outgrowth could be a result of a parasite or cancer, as carotenoids are known to function in cancer suppression (Hill 1999). Our results indicate that pools with greater coloration exhibit more signs of disease, supporting a tradeoff between immune function and coloration.

One possible explanation of this tradeoff is that a more colorful fish cannot successfully fight disease. Alternatively, the handicap hypothesis of sexual selection predicts that males able to thrive while displaying intense orange color and signs of illness will increase their attractiveness to mates (Cotton 2004). Through honest signaling, a male can increase his sexual fitness by indicating to females that he is fit enough to survive even with an obvious disease. Though difficult to apply in this population with very

similar sexes, successful sexing of *A. aeneus* may shed light on this handicap hypothesis.

Predation is a potential disadvantage of increased color intensity not quantified in this experiment. The location of the coloration on *A. aeneus* potentially limits predation pressure to diurnal aquatic predators. Future studies could examine the density of larger fish and decapod crustaceans as a potential explanation for differences in coloration.

The disadvantages and advantages of coloration interact in complex ways to determine the optimal coloration. As the environment shifts spatially and temporally, individuals must respond with changes in their phenotype to maintain their fitness. Coloration is one example of the tradeoffs that organisms must make to survive in their environment. Understanding the pressures that align to form these tradeoffs is essential for understanding the patterns and wonders that surround us in nature.

ACKNOWLEDGMENTS

We would like to thank Erica Deinert for sharing her expertise on the ecology of Corcovado and for aid in locating relevant literature.

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Table 1. Correlation matrix between average saturation, tumor level, average canopy cover, and Heliconiaceae level of the eight pools. Only average saturation and tumor level are correlated.

	<u>Mean Saturation</u>	<u>Tumor Level</u>	<u>Mean Canopy Cover</u>	<u>Heliconia Level</u>
<u>Mean Saturation</u>	1	-	-	-
<u>Tumor Level</u>	0.75	1	-	-
<u>Mean Canopy Cover</u>	0.22	-0.24	1	-
<u>Heliconia Level</u>	-0.35	-0.54	0.48	1
<u>Population Estimate</u>	-0.01	-0.29	0.62	0.01

Table 2: In the presence of conflicting pressures acting on *A. aeneus* color, the disadvantages to intense color, the presence of diseases and limited carotenoids, should decrease color, while the advantages to color, conspecific social signaling and low visibility, should increase color intensity.

<u>Pressure</u>	<u>Prediction</u>	<u>Explanation</u>	<u>Result</u>
Visibility	Reduced visibility increases optimal color intensity	More pigment is needed to obtain same level of perceived color	No relationship between canopy cover and color intensity
Social Signaling	Increases optimal color intensity	As size of community increases, there is more competition for mate selection and dominance	No relationship between population size and color intensity
Diseases	Decreases optimal color intensity; Brighter fish should show more signs of disease	Allocating carotenoids to pigmentation rather than immune system increases vulnerability to disease	High presence of outgrowths in a pool was strongly correlated with more intense color ($r^2 = 0.55$, $P = 0.03$; Fig 2)
Limited Carotenoids	Decreases optimal color intensity	Production of pigment limited by resource availability; Pressure of tradeoffs increases	No relationship between presence of <i>Heliconia</i> and color intensity

Appendix A

Table 3. Data collected on eight pools in the Rio Camarones riverbed during the dry season in Corcovado National Park, Costa Rica.

<u>Pool</u>	<u>Population</u>	<u>Length (m)</u>	<u>Depth (m)</u>	<u>Width (m)</u>	<u>Average Color</u>	<u>Tumor Level</u>	<u>Heliconia Level</u>	<u>Average Canopy</u>
	<u>Estimate</u>				<u>Saturation</u>			<u>Cover</u>
1	567	30.34	0.6	3.6	28.0	2	2	16.38
2	358	22.13	0.6	5.0	36.7	3	1	13.52
3	303	14.23	0.4	4.4	29.0	1	3	22.36
4	517	23.53	0.4	3.3	16.5	2	3	18.98
5	88	9.35	0.1	1.6	52.3	4	2	11.96
6	55	6.83	0.2	2.0	22.6	3	2	6.24
7	115	13.95	0.1	1.4	10.5	1	3	7.28
8	280	10.10	0.3	4.5	19.3	2	1	4.42

MORPHOLOGY AND STRUCTURAL HABITAT USE OF *ANOLIS* LIZARDS IN A MAINLAND COMMUNITY

MADILYN M. GAMBLE, BENJAMIN J. KESSLER, MADELINE K. KREHER, AND AMY VAN SCOYOC

Faculty Editor: Matthew P. Ayres

Abstract: Interspecific competition and sexual selection are two potential forces influencing evolutionary divergence in structural habitat use. The interspecific competition among *Anolis* lizards for niche space on the Greater Antilles Islands has led to the convergent evolution of lizard morphology into specific ecomorphs related to habitat in a way not observed on the mainland. Sexual selection and resulting sexual dimorphism can also lead to complex phenotypes and partitioned habitat use between the sexes of a species. We investigated how structural habitat use is related to complex phenotypes by comparing morphological characteristics of 6 mainland anole species at the La Selva Biological Station, Costa Rica, to habitat use and the common ecomorph categories found in the Greater Antilles. We also examined whether sexual dimorphism and divergent habitat use existed within this mainland population of *Anolis limifrons*. We found no evidence that Greater Antillies ecomorph categories exist on the mainland, nor did we find evidence for sexual dimorphism in *A. limifrons*. However, we found that perch height in female *A. limifrons* decreased with greater pelvic girdle width, while male perch height was positively correlated with body size. We propose that because females exhibiting a wider pelvic girdle may have a higher reproductive fitness, they may be better competitors for the desired foraging territories closer to the ground. For males, a larger body size on higher perches may suggest that larger males are outcompeting smaller males for those preferred display sites.

Key words: *Anolis limifrons*, *Anolis*, lizard, ecomorph, habitat use, sexual dimorphism

INTRODUCTION

The phenotypic diversity of organisms in nature is shaped by their interactions with the environment. Animals best adapted to their structural habitat will tend to be favored by natural selection. Interspecific competition and sexual selection are two potential mechanisms that may drive the evolution of species and divergence in structural habitat use. Yet, since such morphological adaptations are still subject to developmental biomechanical constraints, predictable morphotype categories might arise independently in communities of similar species experiencing similar structural habitats (Beuttell and Losos 1999; Pounds 1988).

The expansion of the genus *Anolis* (small, insectivorous lizards) to the Greater Antilles Islands and resultant adaptive radiation and niche partitioning within those islands has

produced such categories of predictive habitat morphologies (ecomorphs) (Losos 1992). Genetic evidence shows that ecomorphs are the product of convergent evolution. That is, those phenotypes occupying similar habitats (i.e., tree-crown ecomorphs on two separate islands) are more distantly related than their morphologically dissimilar counterparts in separate niches on the same island (Langerhans et al. 2006; Losos 1992). Though it is evident that structural habitat use and morphological adaptations are predictable within the Greater Antilles Islands, evidence for similar ecomorph categories on the mainland of Central America has been notably absent (Irschick et al. 1997).

Sexual selection, either by male-male competition or female choice, can also drive morphological adaptations to habitat use by providing intraspecific pressures. Due to biomechanical constraints, the sexual selection for

one trait often leads to correlated selection, where conglomerations of other traits are inherited with those that are specifically under selection. This selection for a complex phenotype can lead to sexual dimorphism and niche partitioning between the sexes. Such sexual dimorphism is known to occur within populations of *Anolis lemurinus* in the Cayos Cochinos Archipelago of Honduras. Interestingly, these populations do not show differences in habitat use between the sexes (Logan et al. 2012). Conversely, divergence in habitat use between the sexes in *Anolis limifrons* has been documented, while evidence of sexual dimorphism is lacking (Talbot 1979).

To understand how structural habitat uses, arising from interspecific competition and/or sexual selection, drive the complex phenotypes observed in anoles on the mainland, we investigated morphological characteristics and habitat use among species of *Anolis* in primary forest of the La Selva Biological Station, Costa Rica. We took measurements on habitat features and morphological characteristics of 6 different anole species and compared them to the standard ecomorphologies of anoles found in the Caribbean.

We also looked for evidence of sexual dimorphism and differential habitat use between sexes within one anole species, *Anolis limifrons*.

METHODS

We collected our data on February 13-15, 2012 in a primary forest ecosystem of La Selva Biological Station, Costa Rica. We recorded habitat data on every *Anolis* lizard we encountered ($n=46$), noting species, time of day, perch height, perch width (measured along the plane tangent to the lizard), type of perch (leaf, trunk or branch), percent vegetation, and percent canopy cover. To measure percent vegetation we estimated the percent of a 1 m cube centered about the perch occupied by plant material. We used a spherical densiometer to measure the percent canopy cover at each location. We also measured sex, snout-to-vent length (SVL), mass, tail length, jaw width, jaw length, forelimb length, hindlimb length, pectoral girdle width, pelvic girdle width, toe pad length, toe pad width with dial calipers and a Pesola scale.

Data Analysis

We qualitatively compared the morphology of the mainland anoles to what would

Table 1. Average morphological characteristics of *Anolis* lizards in La Selva National Park (means \pm SE).

Lizard Species (n)	SVL (mm)	Tail Length (mm)	Mass (g)	Hind limb length (mm)	Fore limb length (mm)	Jaw Length (mm)	Jaw Width (mm)	Toe Pad Length (mm)	Pectoral Girdle (mm)	Pelvic Girdle (mm)
<i>A. biporcatus</i> (1)	92.1	230.0	26.0	39.5	25.9	16.5	15.1	4.7	14.0	7.8
<i>A. capito</i> (1)	79.9	150.3	10.0	48.7	26.8	13.2	13.3	3.6	11.2	4.0
<i>A. humilis</i> (5)	34.3 \pm 2.7	54.2 \pm 1.3	1.1 \pm 0.3	19.4 \pm 1.5	9.6 \pm 0.8	6.5 \pm 0.3	6.0 \pm 0.4	1.9 \pm 0.3	5.4 \pm 0.6	2.7 \pm 0.4
<i>A. lemurinus</i> (1)	46.7	86.1	2.15	23.7	14.1	10.0	8.0	2.3	6.9	4.5
<i>A. limifrons</i> (28)	35.7 \pm 0.7	73.6 \pm 2.3	0.9 \pm 0.1	19.5 \pm 0.8	10.6 \pm 0.3	6.6 \pm 0.2	5.8 \pm 0.1	1.9 \pm 0.1	4.7 \pm 0.2	2.4 \pm 0.2
<i>A. lionotus</i> (1)	64.0	109.9	6.0	35.8	20.3	13.5	10.2	3.0	-	-

RESULTS

Table 2. Average habitat characteristics of *Anolis* lizards in La Selva National Park (means \pm SE)

Lizard Species (n)	Perch Height (cm)	Perch Width (mm)	% Canopy Cover	% Vegetation
<i>A. biporcatus</i> (1)	190	1.7	74	15
<i>A. capito</i> (1)	195	4.0	-	5
<i>A. humilis</i> (7)	45 \pm 8	3.8 \pm 1.2	98 \pm 0.5	16 \pm 4
<i>A. lemurinus</i> (2)	63 \pm 23	27.8 \pm 22.2	96 \pm 0.1	11 \pm 9
<i>A. limifrons</i> (34)	126 \pm 54	3.6 \pm 0.5	95 \pm 0.5	11 \pm 1
<i>A. lionotus</i> (1)	190	2.2	41	3

have been expected if they fit the Greater Antillean ecomorph categories (Losos 1992). We used a Principle Components Analysis (PCA) on the morphological data gathered for *Anolis limifrons*. We used the first two principle components generated from this analysis (PC1 and PC2) in a biplot by sex to look for visual evidence of sexual dimorphism in morphology and habitat use in *A. limifrons*. Since PC1 accounted for body size variables and PC2 was determined mainly by body shape variables, we used ANOVAs between PC1 and sex and PC2 and sex to look for sexual dimorphism in body size and body shape respectively. We also used a PCA on habitat data of *A. limifrons* to determine whether habitat use differs between the sexes.

We performed a correlation analysis on the relationship between pelvic girdle width and perch height in females since it is known that female reproductive success is influenced by pelvic girdle width (Michaud and Echternacht 1995). We also performed a correlation analysis on the relationship between PC1 from the morphological PCA and perch height in males since male body size is indicative of fitness (Laileaux et al. 2004). We analyzed our results with JMP 9 (Cary, NC)

We collected data on a total of 46 anoles representing 6 different species and obtained morphological data for 37 of these individuals. Thirty-five of the lizards we measured were *Anolis limifrons*.

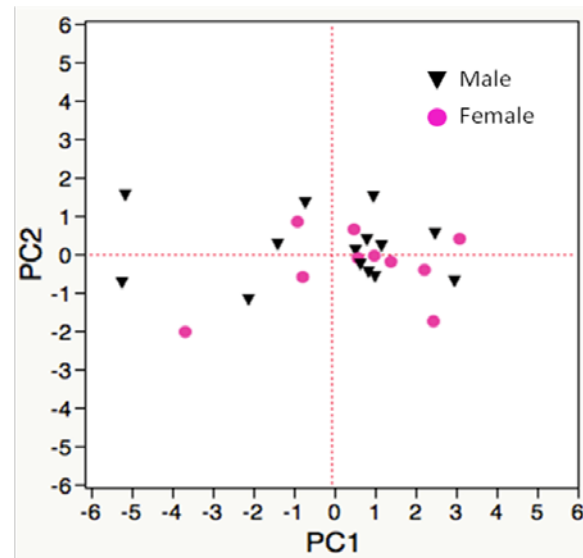


Fig 1. The biplot of PC1 and PC2 from the principal component analysis of morphological data shows no difference in body size or body shape between the sexes.

Though we collected limited morphological data for most anole species, we found no evidence that anoles in La Selva fit into any of the ecomorph categories that exist on the Car-

ibbean islands (Table 1, Table 2). Furthermore, habitat partitioning did not appear to follow the strict vertical boundaries found for ecomorphs in the Greater Antilles (Losos 1992).

The PCA of morphological data showed that PC1 explained 59% of the variation in *Anolis limifrons*, and PC2 explained an additional 11% of the variation. The PC1 loading matrix showed that it was strongly positively correlated with overall lizard size (Table 3). The PC2 loading matrix showed it was most positively related to toe pad length, hind limb length, and SVL, and most negatively related to jaw length, and thus we interpreted PC2 as an indication of overall lizard shape (Table 3). Males and females were essentially identical with respect to PC1 and PC2 ($F_{1,22}=0.73$, $P=0.40$ for PC1; $F_{1,22}=1.42$, $P=0.25$ for PC2; Fig. 1).

From the PCA of habitat data, PC1 explained 47% of the variation, and PC2 explained an additional 25% of the variation (Table 4). Males and females were also the same with respect to their placement on the habitat biplot of PC1 and PC2 (Fig. 2).

Females with wider pelvic girdles tended to have lower perches than those with narrower pelvic girdles ($t=-2.08$, $p=0.076$, $r=0.62$; Fig. 3). There was a correlation between PC1 and perch height in male lizards ($t_{1,12}=2.20$, $p=0.048$, $r=0.54$; Fig. 4) but not in female lizards ($t_{1,8}=-0.38$, $p=0.71$, $r=-0.13$), though pelvic girdle width positively correlates with size ($t_{1,15}=3.13$, $p=0.007$, $r=0.63$).

DISCUSSION

We evaluated two possible relationships between complex phenotypes and the structural habitat in anoles. Interspecific competition could drive anoles into different habitats with unique selective pressures. Alternatively, sexual selection might influence the

Table 3. Morphology PCA loading matrix for PC1 and PC2.

Morphological feature	PC1	PC2
SVL	0.810	0.320
Tail Length	0.765	-0.161
Mass	0.887	-0.149
Hindlimb length	0.662	0.391
Forelimb length	0.797	-0.119
Jaw Length	0.717	-0.543
Jaw Width	0.752	-0.172
Toe Pad Length	0.719	0.487
% Variance	59	11

related selection results in morphologies that

maximize fitness in a structural environment distinct from the other sex.

Though we collected limited data for species other than *Anolis limifrons*, no anole species in our mainland study site appeared to match the morphology of the ecomorphs found on the Greater Antilles in the Caribbean (Losos 1992). Other investigations have reported similar conclusions (Schaad and Poe 2010, Irschick et al. 1997).

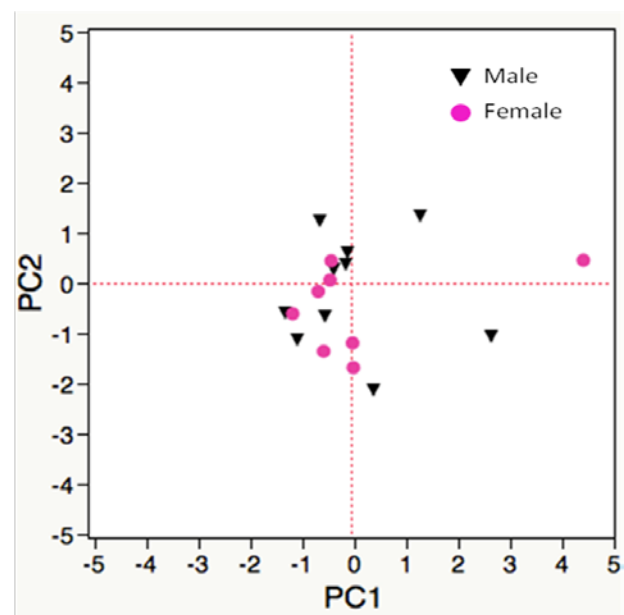


Fig 2. The biplot of PC1 and PC2 from the principal component analysis of habitat data shows no difference in habitat use between the sexes.

This might suggest that there is only weak habitat partitioning among species on mainland Central America. Another possibility is that anoles partition habitat differently on the mainland. Irschick et al. (1997) found that morphological variation correlated most with perch diameter in the Caribbean, while perch

Table 4. Habitat PCA loading matrix for PC1 and PC2.

Habitat	PC1	PC2
Perch height	-0.460	0.813
Perch width	0.768	-0.091
% Vegetation	0.658	0.580
Canopy cover	-0.795	-0.079
% Variance	47	25

height was more important in mainland anoles. A wider survey of anoles within Central America could uncover mainland ecomorphs distinct from those that characterize the Greater Antilles.

Sexual selection, either through female choice or male-male competition, resulting in correlated selection could also explain complex phenotypic relationships with the structural environment for a species. However, *A. limifrons* exhibited neither sexual dimorphism nor differential use of the habitat between sexes, making this mechanism improbable for our system.

Between the sexes, however, univariate correlation analyses uncovered differential relationships between morphology and habitat. Females with wider pelvic girdles perched higher. Though we are unable to fully explain this pattern, it is notable that pelvic girdle width is a particularly important constraint to egg laying for female lizards (Castilla and Bauwens 2000, Michaud and Echternacht 1995). Furthermore, Andrews (1971)

and Talbot (1979) found that females prefer lower perches to maximize foraging while males tend to perch higher. If wide pelvic

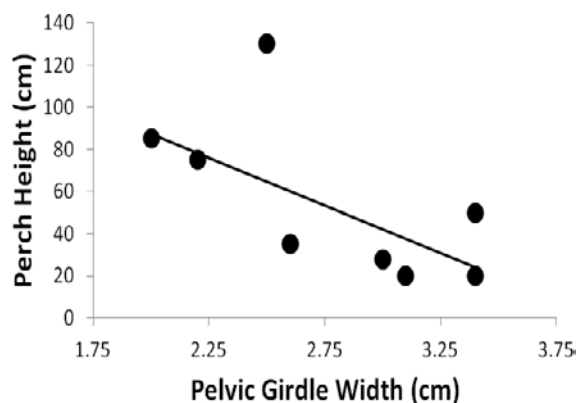


Fig 3. Females with wider pelvic girdles tended to perch lower than those with narrower pelvic girdles.

girdles indicate high fitness in females, then perhaps these fitter females are better competitors for the desired low perch territories.

Fitness in males is better captured by body size than pelvic girdle width since males do not face the same reproductive constraints as females. Instead, body size largely determines male fitness as larger males fare better

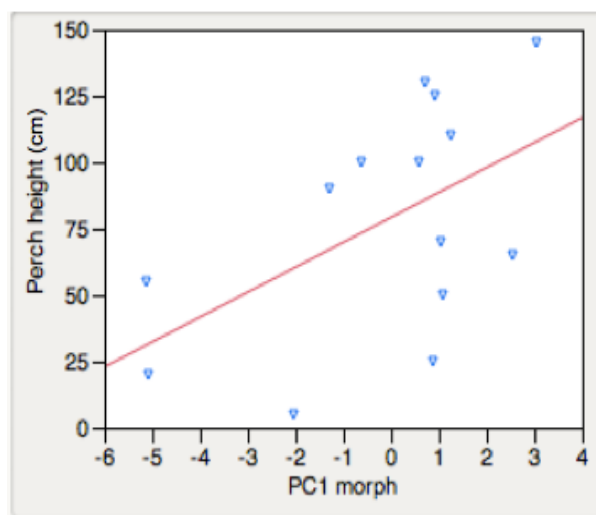


Fig 4. Males with bigger body size (indicated by larger morphology PC1 values) perched higher than males with smaller body sizes.

in territorial encounters (Lailvaux et al. 2004).

Our results indicate that larger males tend to perch higher. If males prefer higher perches as Andrews (1971) and Talbot (1979) suggest, then larger, fitter males might more effectively compete for these higher territories, perhaps in order to better display themselves to females.

The convergent evolution of Greater Antillean anole ecomorphs contributes to the argument that evolution follows predictable trends. However, the lack of such predictable trends within the same genus on the Central American mainland emphasizes that similar selection pressures do not always push a phenotype towards a deterministic end. This highlights the complexity of evolutionary mechanisms and the enigmatic interactions between constraint and contingency in the evolution of species.

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DO *DENDROBATES PUMILIO* BEHAVIORALLY THERMOREGULATE?

MUHAMMAD HEMAYAT R. CHOWDHURY

Faculty Editor: Matthew P. Ayres

Abstract: Maintenance of body temperature within a suitable range and maintaining hydration are critical to the survival of tropical amphibians in the dry season. Understanding the thermobiology and moisture requirements of amphibians is relevant to their conservation. Although the small territorial frog, *Dendrobates pumilio*, is abundant in lowland tropical forests during the wet season, they become far more scarce and are restricted in distribution during the dry season. I hypothesized that territory selection by *D. pumilio* would indicate whether it thermoconformed or thermoregulated and that it would choose the wettest possible habitats so as to minimize the rate of water loss through evaporation. I sampled the body temperature of *D. pumilio* ($N_{\text{total}} = 30$) and recorded the soil, leaf litter surface and air temperature in their territories to investigate how their body temperatures change in relation to their environment. I also rated soil moisture categories and to determine the soil moisture levels most preferred by *D. pumilio*. I found that the body temperature of *D. pumilio* varied significantly from the metrics of environmental temperature, but did not prove that *D. pumilio* behaviorally thermoregulates. I also found that *D. pumilio* preferred habitats of intermediate soil moisture ($\approx 35\%$). More knowledge is needed to anticipate the effects of changes in climate and forest type on *D. pumilio*.

Keywords: *Dendrobates pumilio*, behavioral thermoregulation, thermoconformity, evaporative cooling

INTRODUCTION

Temperature is one of the most important factors that influences species behavior and distribution. Temperature fluctuations are particularly important for ectotherms that are more dependent on the temperature of their environments to maintain optimal body temperature. In addition, ectotherms such as frogs are also dependent on suitable hydric environments as they are susceptible to dehydration via water loss from their permeable skin. Water loss increases with temperature and therefore a tradeoff may arise between territories that are warm enough to sustain performance and those that are humid enough to keep the frog hydrated. This tradeoff becomes more critical in the dry season when tropical forest frogs must find a way to maintain both moisture level and an ideal body temperature in a hotter and drier environment.

Ectotherms can reach ideal body temperature through either behavioral thermoregulation or thermoconformity. Behavioral thermoregulators maintain a relatively narrow variance of temperature throughout the day in the face of temperature fluctuations in their environment. Thermoconformers track the temperature of their environments throughout the day and are therefore more dependent on a stable thermal environment. (Logan, M. L. *pers. comm.*) However, many tropical ectotherms have surprisingly low upper thermal tolerances and are negatively affected when temperatures rise too high (Huey et al. 2009).

If a species is a thermal conformer and has evolved to exploit a very narrow temperature range, such as in tropical lowlands, it may be particularly vulnerable to increased variation in environmental temperature (Huey et al. 2009). In addition to not being able to adjust their temperature to avoid overheating, they would also have a

lower upper critical thermal limit as they had evolved to take advantage of a stable temperature regime (Duarte et al. 2011). The effects of climate change could therefore drastically alter environmental conditions and jeopardize the survival of numerous thermoconforming tropical frog species.

The Strawberry Poison Dart Frog, *Dendrobates pumilio*, is a small, highly territorial terrestrial frog that inhabits forest edge and secondary forest habitats in the lowland tropical rainforests of Costa Rica. Although *D. pumilio* can be found in abundance during the rainy season, they are far scarcer in the dry season (Logan, M. L. *pers comm.*). To better understand the thermobiology of this enigmatic species in relation to their thermal and hydric environment I sampled the surface temperature of *D. pumilio* ($N_{\text{total}}=30$) as well as the moisture level and environmental temperatures in their microhabitats. I hypothesized that *D. pumilio* would inhabit the wettest territories available to compensate for evaporative water loss and that they would be thermoconformers.

METHODS

I sampled 30 *D. pumilio* near the station clearings and trails of the La Selva Biological Station in Costa Rica on the 14th and 15th of February 2012. Surveys were conducted from 0800-1200 and 1430-1800 on Day 1 and from 0530-700, 0745-1200 and 1430-1800 on Day 2. I used a Raytek Infrared Temperature Gun to measure the external body temperature of each *D. pumilio*, as well as the soil, leaf and air temperature at breast height in the shade within a 1.5 m radius of each *D. pumilio*. Soil and leaf temperature were recorded by measuring the temperature of the soil

underneath the leaf litter and the upper surface of the leaf litter, respectively. I measured air temperature at breast height by measuring the surface temperature of a white cotton ball that I had suspended at breast height and allowed to come to thermal equilibrium with the environment. During sampling on Day 2, I also recorded the highest and lowest temperatures at ground level (both above and under the leaf litter) by scanning the temperature of all surfaces within a 1.5m radius of each *D. pumilio*.

To estimate the range of temperatures available to *D. pumilio*, I haphazardly recorded soil, surface and air at breast height temperatures at 15-minute intervals from 1430 onwards on Day 1 and throughout the sampling time on Day 2 at sites that either were, or appeared to me that they could have been, territories held by *D. pumilio*. At each of these sites I recorded the highest and lowest temperatures at ground level (as described above) throughout sampling time on Day 2. I rated soil moisture (as a proxy for humidity) on a five-point-scale at each of these sites on both days. A rating of 1 on the five-point scale corresponded to extremely dry soil and a rating of 5 corresponded to extremely damp soil. I collected surface soil samples ($\approx 10\text{cm}^3$) from a subset of 11 of these sites. I weighed the samples, dried them in a Precision Gravity Convection Oven set at 60°C, and reweighed them to determine the percentage of soil moisture.

Data Analysis

I plotted frog temperature relative to air, surface and soil temperature over the range of temperatures over the course of the day (20-30°C). I compared the slope of the regression to the theoretical expectation of 1

for a perfect thermal conformer. I plotted the frequency of samples where frogs were expected to be found against the frequency of samples where frogs actually were found across the soil moisture categories. I ran a Pearson's Chi square analysis on the two categories (2 and 3) where expected values were sufficiently high. All statistical analyses were conducted using the JMP 9 statistical package (SAS Institute, Cary, NC).

RESULTS

Frog, soil, surface and air temperature all increased the morning and peaked between 1200 and 1400 (Fig. 1). The highest temperature reached by a frog was 26.3°C while the lowest value was 21.8°C.

Frog temperatures tracked soil temperature quite closely, but frog temperatures were buffered relative to their environment by $\approx 1^\circ\text{C}$ at each end of the experienced environmental temperatures (Fig. 2A; slope = 0.723 ± 0.088 , $t = 3.15$, $df = 28$, $P = 0.0039$).

The average soil moisture for samples from categories 2, 3 and 4 were 23%, 35% and 45%, respectively. Compared to the null model of no habitat discrimination, frogs were significantly more likely to be found in sites with soil moisture ratings of 3 and less likely to be in sites with a moisture rating of 2 (Pearson's $\chi^2 = 3.95$, $df = 1$, $P = 0.047$; Fig. 3).

DISCUSSION

D. pumilio body temperature followed the same pattern as environmental temperature over the course of the day, but varied modestly less than their environmental temperature. Because my data could not accurately record for operative

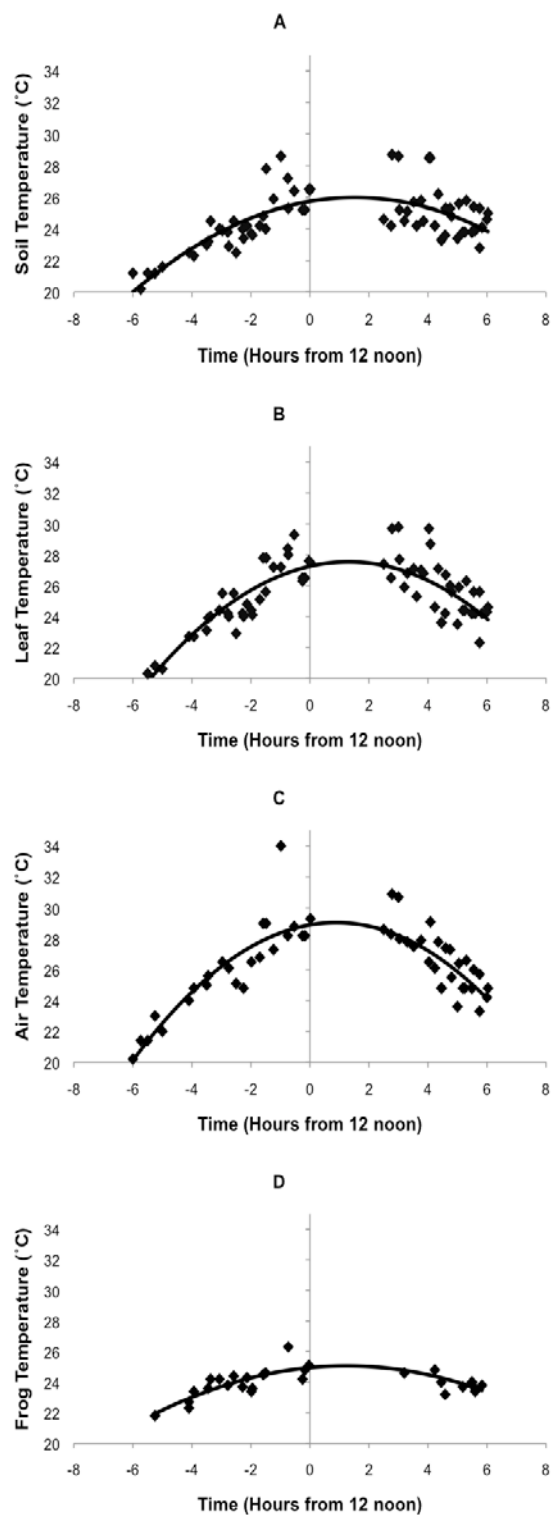


Fig 1. Change in Soil (A), Leaf (B), Air (C) and Frog (D) temperature over the course of a day (centered at 1200) at La Selva Biological Station, Costa Rica.

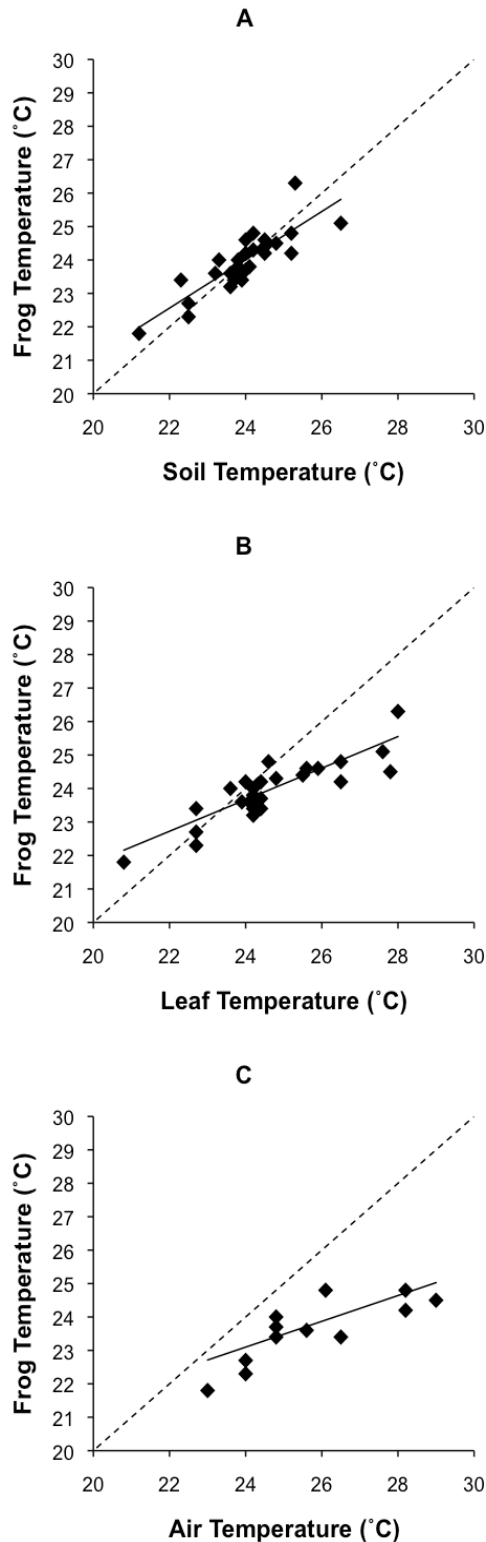


Fig 2. Frog body temperature in relation to Soil (A), Leaf (B), and Air (C) temperature at La Selva Biological Station, Costa Rica. Slopes of the best-fit lines were 0.723 ± 0.088 (A), 0.471 ± 0.055 (B), and 0.386 ± 0.100 (C).

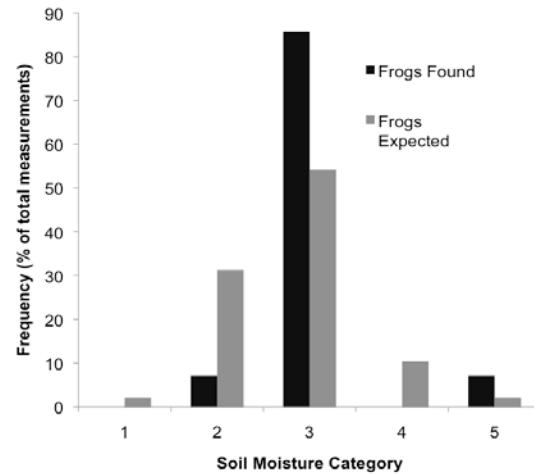


Fig 3. Distribution of frogs found at a range of soil moisture categories in relation to the percentage expected in each soil moisture category. A disproportionately higher number were found in category 3 (approximately 35% soil moisture).

temperatures or correct for the thermal inertia of the frog these results may be misleading. My data is therefore consistent with the hypothesis that *D. pumilio* is a thermoconformer.

Data from frog abundance in territories with varying soil moisture percentages suggests that *D. pumilio* prefer territories with an intermediate level of soil moisture (about 34.6%). This behavior ran counter to the expectation that an amphibian that is vulnerable to dehydration, particularly in the dry season, would choose the wettest territories available. If *D. pumilio* are not selecting the wettest habitats, but selecting for a moderate level of humidity, factors other than water loss, such as risk of fungal infection in moist habitats, may be more important in choosing territories. This habitat specificity may make *D. pumilio* vulnerable in the event of a hotter and drier environment resulting from climate change.

If *D. pumilio* are basically thermoconformers and are restricted to a narrow range of soil moistures, they may be more

vulnerable to climate change than would otherwise be the case. This may partially explain the observed decline of *D. pumilio* in La Selva Biological Station (Whitfield et al. 2007). Aggregation of *D. pumilio* to restricted areas may make them vulnerable to parasites, fungi and other pathogens, putting them at risk of further decline (Donnelly and Crump, 1997). To survive in the event of such abiotic stresses frogs would either have to rapidly adapt or move to cooler wetter locations at higher elevations. The absence of frogs from the lowlands could lead to biotic attrition, and if no new species move in to fill their niche this could have additional compounding effects on the food web and ecology of the area (Colwell et al. 2008).

With 41% of all species being listed as threatened, amphibians are the most globally vulnerable group of all vertebrates (Wake and Vredenburg, 2008). If *D. pumilio*, one of the more common species at La Selva, is under stress it is likely that other species are under pressure as well. In the event of environmental change moisture specific organisms would have fewer habitats to choose from, ectothermic thermoconformers would face greater extremes of temperature that they may not be able to cope with, and thermoregulators would have to work harder to maintain suitable body temperatures. If populations of these organisms decline it is likely that there would be far reaching effects on the ecology of the system. It is therefore essential to develop a better understanding of species thermobiology to predict or possibly prevent further degradation of the earth's biodiversity.

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HOW *CHOLOEPUS HOFFMANNI*'S POIKILOTHERMY PRESERVES THE SLOTH NICHE

ROBIN A. COSTELLO AND JESSE T. RIEB

Faculty Editor: Matthew P. Ayres

Abstract: Organisms generally employ one of two main thermoregulatory strategies. Thermoregulators actively control their body temperature, while thermoconformers allow their temperature to conform to ambient temperature. However, some animals use a combination of these two strategies. The two-toed sloth, *Choloepus hoffmanni*, acts as a facultative poikilotherm, allowing its body temperature to conform to ambient temperature over a much wider range of temperatures than other mammals. We investigated potential energy savings as an explanation for the evolution of facultative poikilothermy in the two-toed sloth. We observed the breathing rate of a two-toed sloth to determine the relationship between its breathing rate and ambient temperature. We also modeled a theoretical sloth that behaved as a strict homeotherm in order to quantify the energy saved through facultative poikilothermy. We found that as a facultative poikilotherm, the sloth's breathing rate increases linearly as a function of temperature, while a homeotherm's breathing rate would increase at colder temperatures. Poikilothermy allows the sloth to use approximately 30% less energy over the range of temperatures experienced during a typical day. We concluded that the energy savings from facultative poikilothermy are substantial and possibly essential for supporting the folivorous and sedentary lifestyle of a sloth.

Keywords: breathing rate, *Choloepus hoffmanni*, energy savings, facultative poikilotherm, homeotherm, thermoregulation

INTRODUCTION

Even in tropical climates, organisms do not live in an environment of constant temperature. Organisms have evolved different thermal strategies to cope with this variable environment, most conspicuously endothermy vs. ectothermy. Whether or not an organism maintains its body temperature through metabolic processes has implications for its energetic requirements. A thermoregulating endotherm requires a higher metabolic rate when the environmental temperature is lower or higher than the organism's thermal neutral zone (Fig 1). On the other hand, a thermoconforming ectotherm has a metabolic rate that scales with environmental temperature (Fig. 2). As the environment and organism simultaneously warm, the ectotherm's metabolic rate increases. Homeotherms, organisms that maintain a constant internal temperature, require a higher metabolic rate

than poikilotherms, organisms that allow their internal temperature to conform to that of the environment, at all but the warmest temperatures. While the conventional wisdom is that endotherms are homeotherms and ectotherms are poikilotherms, organisms fall along a spectrum of thermoregulatory

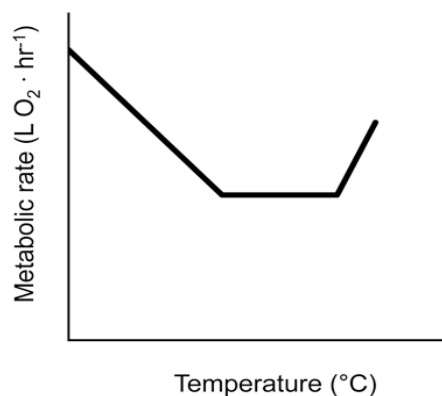


FIG 1. The effects of temperature on the metabolic rate of an idealized endothermic homeotherm. An endothermic homeotherm has the lowest metabolic rate in the thermal neutral zone. Below the lower critical temperature and above the upper critical temperature, the metabolic rate increases.

strategies. For example, bumblebees (*Bombus spp.*) achieve homeothermy by generating heat with their flight muscles.

Another organism that has evolved an unconventional thermal strategy is the sloth. Sloths, which are notoriously slow moving, have apparently evolved low activity levels that decrease their average daily metabolic rate (ADMR). It has also been found that their basal metabolic rate (BMR), the metabolic rate at rest, is low compared to that of other similarly sized mammals (Scholander et al. 1950). This lower metabolic rate could have evolved as a mechanism for subsistence on a nutritionally-poor diet of leaves. Alternatively, with low metabolic requirements, sloths do

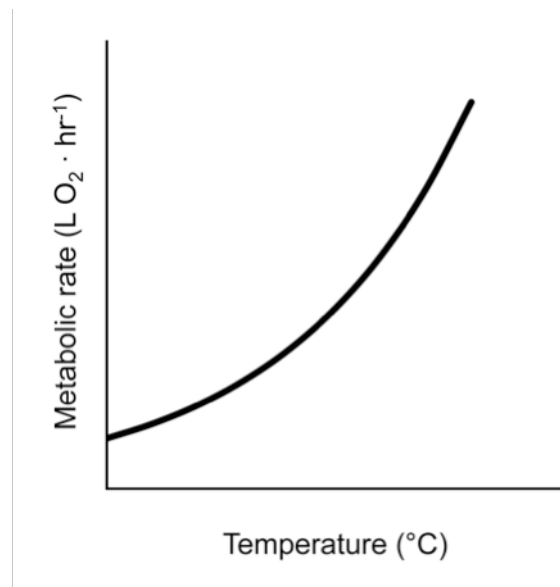


FIG 2. The effects of temperature on the metabolic rate of an idealized ectothermic poikilotherm. As the temperature increases, the metabolic rate of the ectotherm increases exponentially.

not need to continuously forage, allowing them to remain motionless throughout the day and to use crypsis to avoid predators.

Sloths' thermoregulatory strategy, which can be described as facultative poikilothermy, could account for further reductions in metabolic rate. Unlike most mammals, the body

temperature of a sloth can vary by as much as 10°C (Kredel 1928). Through facultative poikilothermy, sloths have evolved a lower lower critical temperature (LCT), the point below which an endotherm must increase their metabolic rate to thermoregulate. Because sloths are a small mammal (on average 4 kg) with long appendages, they have a high surface area to volume ratio. A hypothetical sloth that maintained a constant body temperature would have a high LCT, and would have to increase its metabolic rate to thermoregulate even in the tropics.

Another potential benefit of thermoconforming is that, at warmer temperatures, the digestive rate increases passively with temperature. With increasing temperature, sloths can increase their digestive rate without thermoregulatory metabolic needs. Sloths have been observed to bask in the sun to increase digestive rate, suggesting that this might be a possible explanation for their poikilothermy (Britton and Atkinson 1938).

In this study, we explored the question of why sloths exhibit facultative poikilothermy and investigated a lower LCT as a possible explanation. We hypothesized that facultative poikilothermy would result in a lower LCT in sloths, and that this lower LCT would reduce energy needs by a meaningful amount compared to maintaining a constant body temperature.

METHODS

We measured the breathing frequency of a single two-toed sloth (*Choloepus hoffmanni*) in La Selva Biological Station, Costa Rica, on February 12 – 15, 2012. We observed the sloth between 1600 and 1730 on 12 Feb 2012, between 1245 and 1745 on 13 Feb, between 0600 and 1230 on 14 Feb, and between 0600 and

1230 on 15 Feb. The sloth did not move more than 30 m during our study period. We assume that we were observing the same sloth throughout the study.

To measure breathing frequency of the sloth, one person used binoculars (8x magnification) to count visible breaths (one inhalation and one exhalation). A second person recorded the beginning and end times of the observed breathing. The observer and timer switched roles every two observations to control for observer bias and reduce eye fatigue. To validate the accuracy of our breathing measurements methods, we used a t-test to compare average breathing measurements between observers. We also noted changes in activity level during observations on Feb 13 – 15.

Shaded air temperature data was collected every 5 minutes using a HOBO TidbiT v2 temperature logger (Onset, Bourne, MA). The logger was placed approximately 2 m off the ground on the trunk of the tree where we found the sloth on the first day in a location where it would not receive direct sunlight at any point in the day. We measured sloth breathing frequency over temperatures ranging from 19.6 to 29.6°C. On subsequent days, our sloth was never more than 30 m away from the logger.

Statistical Analyses and Modeling

We calculated breathing frequency by dividing the total breaths observed in each observation period by the duration of the observation. We assigned the temperature preceding the five-minute period in which the breathing observation began to each breathing frequency. We used a linear regression to determine the relationship between ambient temperature and sloth breathing frequency. Regression analysis was performed using JMP 9 software (SAS Institute, Cary, NC).

To determine the energetic benefits of facultative poikilothermy, we modeled a theoretical "homeothermic sloth," an animal that had the same physical properties as an actual sloth but kept its body temperature fixed at 37°C (Fig 3A). We used an estimate of a two-toed sloth's LCT which was based on the shape, size, and thermal conductance of a typical two-toed sloth and a fixed body temperature of 37°C (Porter and Kearney 2009; Fig 3B). We estimated the breathing rate in the thermal neutral zone (at temperatures above the LCT) of our homeothermic sloth as the breathing rate of our observed sloth at the estimated LCT (Fig 3C). We plotted a line through the LCT and the body temperature to determine the rate of increase of the breathing rate below the LCT (Fig 3D). Using this line and the observed relationship between ambi-

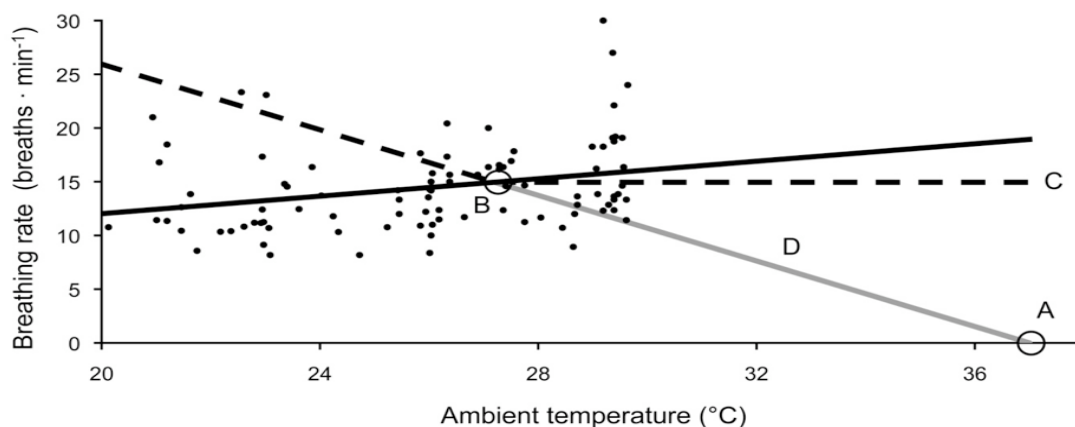


FIG 3. The breathing frequency of our observed sloth (solid black line) and our model of a theoretical homeothermic sloth (dashed line) as a function of ambient temperature. Points are breathing rates measured in the field. We determined the relationship between external temperature and breathing frequency for the theoretical sloth using a body temperature of 37°C (A), an expected LCT at 27.2°C (B), and a metabolic rate of 14.95 in the thermal neutral zone (C). The rate of increase in breathing frequency below the LCT is equal to the slope of the line between the LCT and body

ent temperature and sloth breathing frequency, we compared the breathing rates at the lowest observed ambient temperature.

To compare the daily breathing rates of our actual sloth and theoretical sloth, we integrated the expected breathing rate across the range of temperatures experienced in a day. We used temperature data from 13 Feb 2012. For each temperature measurement, we calculated the expected breathing rate for both observed sloth and theoretical sloth. We multiplied each rate by 5 minutes to extrapolate the total number of breaths until the next temperature measurement. These five minute periods were summed over the entire day to determine the estimated total daily breaths for each of the two sloths.

RESULTS

We found no significant effect of the observer on the observed breathing frequency ($t_{92} = 0.04$, $p = 0.97$). Thus, our methods were consistent across observers.

Shaded air temperature ranged from 19.6 to 30.8°C.

observed the sloth move 27 times in 18 hours (Table 1). Sloth activity included occasional stretching, yawning, scratching, shifting of position, and once moving to a different location. All movements were slow and brief in duration.

The breathing rate of the two-toed sloth increased significantly and linearly with temperature (Fig 3; $r^2_{103} = 0.11$, $p = 0.0005$; $y = 3.9 + 0.41x$). The breathing rate followed this linear relationship across all temperatures measured and did not increase below the predicted LCT for a homeothermic sloth (27.2°C). The breathing rate of our theoretical sloth decreased linearly with temperature at temperatures below 27.2°C ($y = -1.526x + 56.46$). Above the predicted LCT, the breathing frequency was estimated at 14.95 breaths/minute and was not related to temperature.

At the lowest observed ambient temperature, breathing rate was 11.86 breaths \cdot min⁻¹ for the observed sloth and 26.51 breaths \cdot min⁻¹ for the theoretical sloth. The observed sloth used 56% less energy than the theoretical sloth at this temperature.

Across a range of daily temperatures, ob-

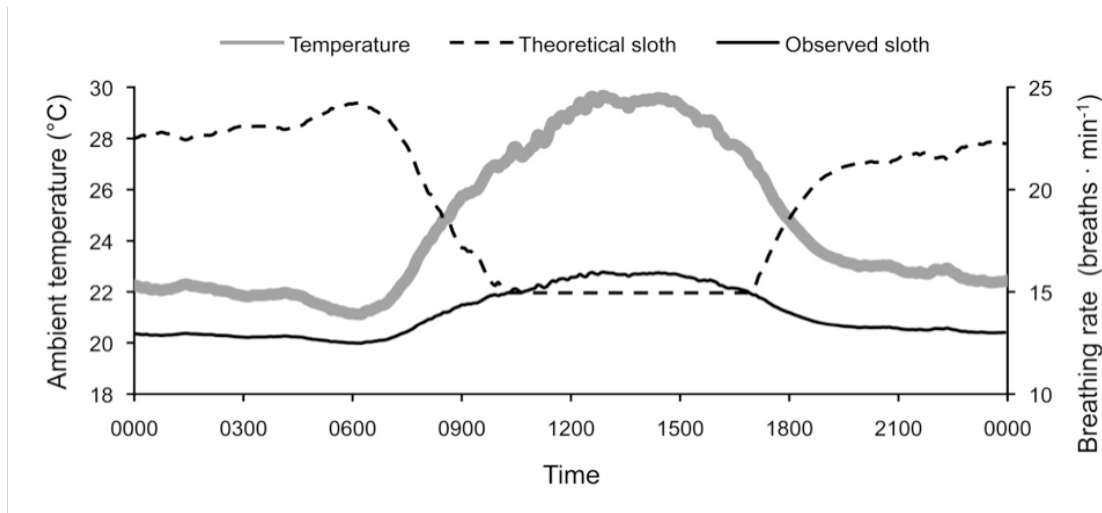


FIG 4. Daily variation of shaded air temperature, predicted breathing rate for the observed sloth, and predicted breathing rate for a theoretical homeothermic sloth on 13 Feb 2012.

served breathing rates varied by a small amount and were positively related to temperature whereas theoretical breathing rates varied by a larger amount and were negatively correlated to temperature (Fig 4). The observed sloth had a lower breathing rate than the theoretical sloth at all temperatures except for the warmest part of the day. We estimated that the observed sloth would breathe 20,027 times in a day and that the theoretical sloth would breathe 28,134 times in a day. Thus, we estimate that the observed sloth used 29% less energy than the theoretical homeothermic sloth.

DISCUSSION

As we expected, the sloth we observed had an extremely low activity level. During our four day study period, the sloth was found on only two neighboring trees, which were less than 30 m apart. However, *C. hoffmanni* is nocturnal and we were only able to observe the sloth during the day. Because of the low diurnal activity rate, our breathing rate measurements are valid estimates of resting breathing rates.

Our data support the idea that the two-toed sloth has evolved a lower LCT. Breathing rate continued to decrease linearly past the theoretical LCT of 27.2°C. If sloths were homeotherms with fixed body temperatures at 37°C, we would expect to see an increase in breathing rate below the LCT. Our finding that the sloth's LCT is lower than 19.6°C is consistent with what we would expect if sloth body temperature were conforming to ambient temperature across a range of approximately 10°C, as previously reported for three-toed sloths (Kredel 1928).

An average basal metabolic rate (BMR) for herbivores has been measured at approxi-

mately 56% of the average daily metabolic rate (ADMR) (Degen et al. 2002). If sloths were able to evolve in such a way that the difference between their ADMR and BMR is negligible, they could reduce their energy needs by up to 44%. Considering this, the additional 29% reduction in daily energy needs through facultative poikilothermy would have a meaningful impact on the biology of the organism. The amount of energy saved through facultative poikilothermy suggests that this adaptation may be a necessary condition for the existence of the niche that the sloth occupies. Without the adaptation of facultative poikilothermy, sloths would likely not be able to obtain sufficient energy by eating leaves alone, and would have had to develop some other adaptation, such as seasonal frugivory as in howler monkeys (Pavelka and Knopff 2004) or increased foraging rates to satisfy their energetic requirements.

When considering thermoregulatory strategies, it is convenient to divide organisms into the discrete categories of homeotherms and poikilotherms. Homeothermic endotherms spend substantial amounts of energy on regulating their internal temperature, and facultative homeotherms have deve-

Table 1. Sloth behavior observed over 18 hours of observation

<u>Activity</u>	<u>Times Observed</u>
Shifting position	11
Scratching	7
Stretching	4
Lifting head	2
Yawning	2
Moving to different location	1
Total Activity	27

loped behavioral or physiological adaptations to regulate body temperature. The abun-

dance of animals that employ one of these two strategies, despite the energetic cost, suggests that homeothermy is adaptive in many environments. However, the evolution of facultative poikilothermy in the two-toed sloth from an ancestral type that was surely an endothermic homeotherm illustrates a rare case of secondarily derived facultative poikilothermy.

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LOAD-VELOCITY TRADE-OFFS AND OPTIMAL FORAGING BEHAVIOR IN *ATTA CEPHALOTES*

NINA B. FRANKEL AND MILO S. JOHNSON

Faculty Editor: Matthew Ayres

Abstract: For eusocial insects, foraging rates should be optimized on the scale of the colony as a whole rather than simply for an individual. Physiological constraints directly affect foraging rates, and other processes involved in the colony's metabolism may shift the optimal foraging strategy. We investigated the tradeoff between load (leaf mass) carried and velocity for the leaf cutting ants *Atta cephalotes* and how ants change load selection over different foraging distances. We found that ants walk slower when they carry heavier loads than predicted for their body size. For five colonies foraging diurnally, we found that ants foraging a longer distance are more likely to carry loads heavier than predicted for their body size. However, when we conducted an experimental manipulation on a colony foraging at night, we found no effect of foraging distance on residual leaf load. We created a model based on the linear relationship we found between velocity and load mass that predicts, for a given ant size and foraging distance, the optimum load an ant should carry to maximize individual foraging rate. We compared our model's predictions to our empirical evidence regarding load size selection at different foraging distances. We concluded that *A. cephalotes* ants forage at a rate below the individual optimum in order to optimize the colony's metabolism. *A. cephalotes* may change their load selection based on distance from their nest only in low trail flow situations, which occur mainly during diurnal foraging.

Key words: foraging rate, optimal behavior, eusocial insects, *Atta cephalotes*, physiological trade-offs

INTRODUCTION

For all organisms, the optimization of metabolic rates has been under strong and continuous evolutionary pressure. As such, we expect the rates at which metabolic processes occur to be optimal within the constraints of the system. For most species, these constraints are to be found inside an individual's body, but for eusocial insects, who live in vast colonies with shared genetic identities, forming what have been called "superorganisms" (Hölldobler and Wilson 1990), the consumption of resources can also be described at the level of the colony as a whole. Therefore, for these species, the optimality of metabolism and the constraints on optimality must be considered at this larger scale, with an individual organism operating as a cog in the metabolic

machinery rather than the machine itself (Burd and Howard 2008).

For leaf cutter ants, an essential component of the colony's overall metabolism is the process of foraging for leaves. *Atta cephalotes*, a leaf cutter species that ranges from Mexico to Brazil, forages along cleared trails radiating outward from the nest, which they use to transport leaf fragments cut from trees back to their nest (Hölldobler and Wilson 1990). The leaves are fed to a fungus in the nest as part of a fascinating obligate mutualism in which the ants cultivate the fungus to later eat the gongylidia, swellings of the fungal hyphae tips (Hölldobler and Wilson 1990). Like other eusocial insects, *A. cephalotes* ants have polymorphic castes that perform different functions for the colony. Within the workers, who primarily forage for leaves, there is variation in individual

ant size and the load of leaf mass carried (Burd 2000). Even ants of the same size exhibit a wide variation in the loads they carry (Burd 2000).

In this study, we examined 1) if there was a tradeoff between load carried relative to body size and velocity during *A. cephalotes* foraging, and 2) the influence of foraging distance on the residual load that *A. cephalotes* individuals cut and carried. We hypothesized that the residual load *A. cephalotes* individuals carried at a given foraging distance would not match the optimal load predicted by a model we developed that maximizes individual foraging rate and would instead be governed by colony-level effects.

METHODS

On 13 February 2012, we located the entrance to an *Atta cephalotes* nest near the La Selva Biological Station in Heredia, Costa Rica. Ten meters from the entrance to the nest, we marked off a 20 cm section of the foraging trail along a concrete path. We measured the time it took for ants carrying leaves back to the nest to cross this 20 cm section of trail. We excluded ants that stopped to communicate and ants that had “hitchhiking” minima on their leaves. After we timed an ant, we used forceps to collect the ant and its leaf (n=35). We weighed each ant’s leaf to the nearest milligram. Ideally, we would have liked to measure ant size by weighing the ants, but we did not have a scale of sufficient precision. We instead measured ant size by measuring ant body length (to the nearest 0.5 mm using a dissecting microscope) from the anterior of the head (excluding mandibles) to the posterior of the abdomen.

To test the effect of foraging distance on load optimization, we located 5 additional *Atta cephalotes* colonies on 14-15 February 2012. We located the nest entrance that leaves were carried back to. We then followed the foraging trail either to the tree at which the ants were foraging, or until we could no longer follow the column with certainty that the ants we saw were returning to the nest. We measured foraging distance from this point to the nest. We collected 10 ants and their loads from each colony. In the laboratory, we again weighed the leaves and measured ant body length (see table 1 for data summary).

We also conducted an experimental manipulation on the night of 15 February 2012 to determine if *A. cephalotes* would select leaf loads differently from locations of two different foraging distances. At 1600 h we removed leaves from a *Nephelium ramboutan-ake* tree and placed leaves (n=30 leaflets) slightly off the *Atta* foraging trail at distances of 20 and 40 m from an *A. cephalotes* nest entrance. This was a different colony than used for our daytime experiments, and was a colony we had previously seen to forage at high density at night. After dark, from 1845-1915 h, we stationed ourselves at the 20 and 40 m foraging distances, waited for *A. cephalotes* to cut loads from the *N. ramboutan-ake* leaves and then collected the ants and their loads that cut these leaves. At both distances, we observed *A. cephalotes* cutting the *N. ramboutan-ake* leaves we had placed there, incorporating these leaves into the foraging trail, and bringing them back towards their nest.

Statistical Analyses

Using data from the first 6 colonies (N=78), we took residual load values from the regression of ant body length and load to obtain a measure of how much more or less an ant is carrying than we would expect for its size based on the linear equation. We treated the controlled night-time experiment as a separate sample and repeated this process independently to obtain load residuals (N=20).

Model Development

We developed a model to predict foraging rate using 2 equations obtained empirically: load as a function of ant body length, and velocity as a function of residual load. See appendix 1 for the derivation of our equation for foraging rate and the resulting equation for optimal load (load at the maximum foraging rate) as a function of body length. We held body length constant at the median length (5.5 mm) and plotted foraging rate as a function of load for 20 and 40 m foraging distances (fig. 4). We plotted optimal load as a function of body length (fig. 1) for comparison to our empirically-obtained relationship between body length and load.

RESULTS

We found that larger ants (body length) carried larger loads ($r^2=0.13$, $df=77$, $P=0.0011$; Fig. 1). Ants that carried loads heavier than the predicted for their size (residual load) were slower ($r^2=0.32$, $df=34$, $P=0.0005$; Fig. 2).

In our daytime test, the ants from colonies foraging further on average carried loads heavier than predicted for their size ($r^2=0.87$, $df=4$, $P=0.020$; Fig. 3). However, in our nighttime test, ants foraging 20 m and 40 m from the nest entrance hole both carried similar loads for their size ($r^2=0.003$, $df=1,9$ $P=0.8$; Fig. 4).

Our model predicts foraging rate for an individual ant based on three inputs: ant body length, load mass, and distance to foraging site. When the other variables are held constant, the foraging rate increases with greater body length, decreases with greater foraging distance, and is parabolic for load mass, with a maximum at a specific optimum load. This predicted optimum load is always half the load that would be predicted to slow the ant's velocity to zero, and the ant's predicted velocity while carrying the optimum load is always half the velocity predicted for the ant carrying no weight.

Table 1. Data collected from 7 *Atta cephalotes* colonies at La Selva Biological station (with standard deviations where appropriate) and the tests they were used in. Colonies 1-6 (N=78) were used to calculate residual load values for further analyses.

Colony ID	Ant Length (mm)	Load (mg)	Distance to Foraging Site (m)	N	Test
1	5.75±0.86	16.18±12.09	NA	34	Speed
2	5.11±0.65	13.56±7.66	25	9	Foraging distance (day)
3	5.65±0.47	12.20±5.65	0	10	Foraging distance (day)
4	5.60±0.61	17±7.04	20	10	Foraging distance (day)
5	5.10±0.82	18.8±4.32	80	5	Foraging distance (day)
6	6.05±0.83	21.70±15.56	47	10	Foraging distance (day)
7	6±1.13	18.5±8.00	20	10	Foraging distance (night)
7	7.15±0.67	20.5±5.85	40	10	Foraging distance (night)

This optimum load increases with greater body length but does not change at different foraging distances. Appendix 1 includes the derivation and details for this model.

DISCUSSION

We found that larger ants, as defined by body length, carried heavier loads, a relationship that has been previously documented (Rudolph & Loudon 1986, Burd 1995). Using this relationship, we found that ants carrying loads heavier than we would predict for their size walked slower than ants carrying less than we would predict for their size. We interpreted this trade-off between heavy loads and fast locomotion as a physiological constraint that may have strong energetic consequences for the optimal foraging rate of an ant.

Based on the assumptions that the relationship between load and velocity is linear for any given ant size and that velocity is a function only of load, our model predicts that maximum foraging rate is higher when an ant is foraging at shorter

distances from its nest. However, for a given ant size, this maximum is always achieved at the same ideal load, regardless of foraging distance. The results of our night-time test may support this model since the load residuals were the same for ants foraging 20 m and 40 m from their nest, but these ants were still foraging well below the optimal load predicted by the model. In fact, the loads carried by our ants were almost always below the predicted ideal load. For example, the mean load carried by a 5.5 mm ant (the median size) was 16 mg, but the model predicts that a 5.5 mm ant would maximize its foraging rate by carrying a 40 mg load. Burd and Howard (2008) noted the same pattern in *Atta colombica*, in which the average worker carries a load of approximately 20 mg despite a projected maximal foraging rate when carrying 35 mg. They propose that this apparent inefficiency can be explained by considering the processing rate of leaves inside the nest, where large loads become a hindrance to the fungus-growing process (Burd and Howard 2008). Thus, though individual ants may be foraging at a

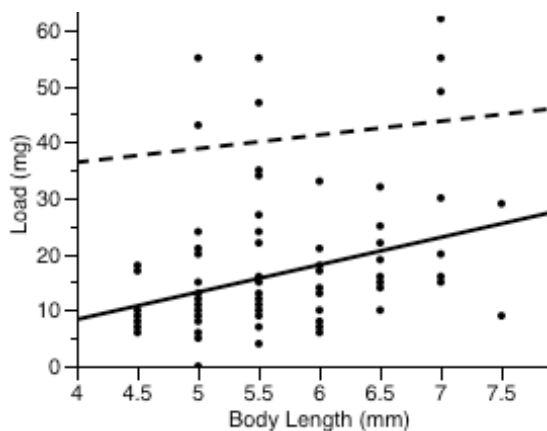


Fig. 1. Body length in *A. cephalotes* ants and the load (mass) they carried (solid line, $\text{Load} = 4.89 * \text{Body Length} - 11.2$.) The dotted line represents optimal load for maximum individual foraging rate as determined by our model. Points represent individual ants.

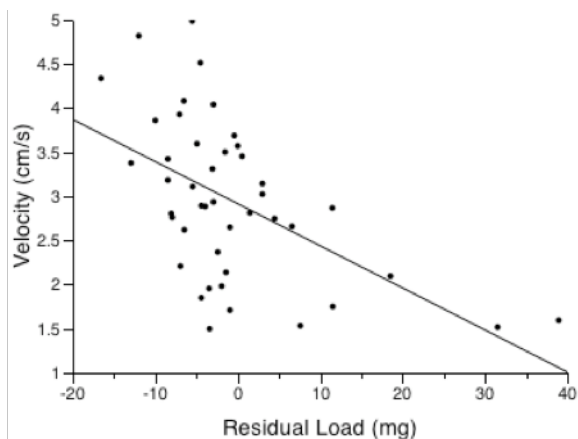


Fig. 2. The residual load carried by an *A. cephalotes* ant (taken from the regression of load and body length, Fig. 1) and ant velocity, measured over a 20 cm course on level cement. $\text{Velocity (cm/s)} = -0.0471 * \text{Residual Load (mg)} + 3.032$.

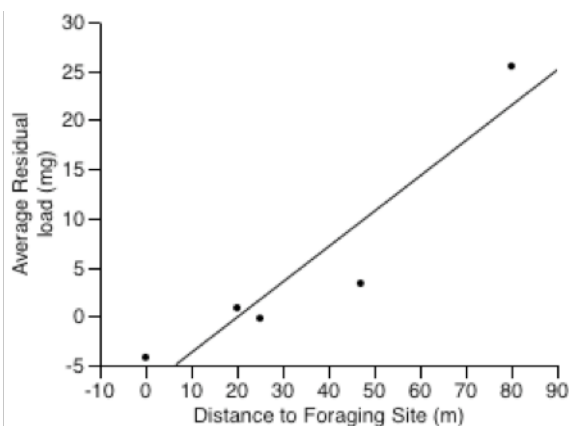


Fig. 3. Distance to foraging area and average residual load. Each point represents one colony at one foraging site and is an average of the residual load from the 5-10 ants collected at the foraging site. Average Residual Load = $0.360 \times \text{Distance to foraging area} - 7.323$.

suboptimal level, the colony viewed as a whole may still be operating at an optimal level (Rudolph & Loudon 1986, Burd and Howard 2008). The importance of leaf processing, as a limitation on load size, also provides a possible explanation for the results of our daytime experiment. Processing rate would be more of a limiting factor at higher foraging rates, which we would expect closer to the nest, both intuitively and from our model.

The truck driver hypothesis (Farji-Brener et al 2011), which details how “heavily-laden” (high load) ants slow down their compatriots, may also provide a logical explanation for how suboptimal individual foraging rates could still potentially maximize overall colony foraging rates. Heavily-laden ants were more common in foraging columns with low trail flow (ants / (100 cm²·s)), where they would slow down fewer sisters with their heavy loads (Farji-Brener et al. 2011). The truck driver hypothesis could also help explain the effect of distance we found in daytime foraging. Since we observed that *A. cephalotes* branch off from their columns to forage at multiple sites, trail flow

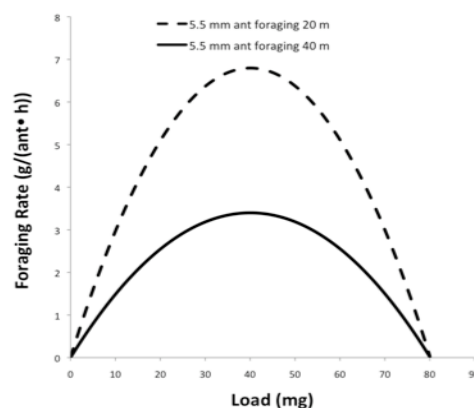


Figure 4. Foraging rate at different loads for a 5.5 mm ant as predicted by our linear equations for ant length, load, and velocity. The dotted line represents a 20 m foraging distance and the solid line represents a 40 m foraging distance. The optimal foraging rates occur at the same ideal load, irrespective of foraging distance (40 mg for a 5.5 mm ant). See appendix 1 for model derivation.

should be greatest near the nest. Ants foraging at greater distances spend a smaller proportion of their foraging time at these high trail flow areas near the nest, so they can carry heavier loads with less effect on their nest mates. In addition, since Farji-Brener *et al.* (2011) found that the percentage of ants that are heavily-laden exponentially decreases as a function of trail flow, their findings could potentially provide an explanation for the apparent contradiction between our daytime and nighttime tests.

Our daytime experiments overall had very low trail flow, while our nighttime experiments had very high trail flow. Since the slope of the exponential decay function is steepest at low trail flow, there would be more of a “traffic” effect, and thus more of our proposed corresponding distance effect, on the size of loads carried during the day. At high trail flow, the slope of the exponential function is nearly zero, so there are less effects of traffic and distance on the load size selection at night. Previous studies conducted at night

at La Selva also concluded that foraging distance had no significant affect on the relationship between load carried and ant size in *A. cephalotes* (Wetterer 1990, 1991). Based on our data and these studies, we conclude that the distance to the foraging site may only change individual load selection behavior in *A. cephalotes* during the day, when trail flow is low.

The trade-off between load and velocity is likely only one piece of the puzzle that is *A. cephalotes* foraging behavior. Certainly, processing rates within the colony and density or traffic effects must play a large role in shaping the ways colonies forage (Burd and Howard 2008, Farji-Brener *et. al.* 2011). For a eusocial insect like *A. cephalotes*, we should not be surprised that workers operate well below the individual optimal foraging rate as long as their behaviors are beneficial to the success of the colony as a whole (Burd and Howard 2008). These diminutive insects, each working as a member of a larger machine, march forth from their nest every day, striving to maximize the overall resource acquisition by their colony, and consequently the fitness of their shared genes.

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APPENDIX 1. MODEL DERIVATION

Variables:

L-Body length (mm)

P-Predicted load (mg)

V-Predicted velocity (m/s)

M-Load (mg)

M*-Optimum load (mg)

D-Distance to foraging site (m)

FR-Foraging rate (mg/s)

We created our models using two equations obtained from regressions of our data:

$$P = 4.885 \bullet L - 11.21$$

$$V = -0.0004755 \bullet (M - P) + 0.02912$$

If we assume that ants make repeated trips with no waiting or cutting time, foraging rate (here in mg/s, but multiplied by a constant to plot kg/h in our figure) is a function of speed, mass carried, and distance to the foraging site:

$$FR = \frac{V \bullet M}{2D}$$

We insert our equations for speed to obtain:

$$FR = \frac{(-0.0004755 \bullet (M - P) + 0.02912) \bullet M}{2D}$$

$$FR = \frac{1}{2D} (-0.0004755 \bullet M^2 + (0.0004755 \bullet P + 0.02912) \bullet M)$$

To check the logic of this model, we found when foraging rate was equal to zero:

$$FR = 0$$

when

$$M = 0$$

or

$$-0.0004755 \bullet M + 0.0004755 \bullet P + 0.02912 = 0$$

so

$$-0.0004755 \bullet (M - P) + 0.02912 = V = 0$$

So foraging rate will be zero if an ant is carrying nothing or if the ant is carrying so much that its speed reaches zero. The logic checks out. Next, we take the derivative of foraging rate and set it equal to zero to find the optimal load that will maximize foraging rate:

$$FR' = \frac{1}{2D}(-0.0009510 \bullet M + (0.0004755 \bullet P + 0.02912))$$

$$FR' = 0$$

when

$$-0.0009510 \bullet M^* + (0.0004755 \bullet P + 0.02912) = 0$$

$$M^* = \frac{(0.0004755 \bullet P + 0.02912)}{0.0009510}$$

So the optimal mass to carry for an ant of given body length is independent of the distance to the foraging site. By inserting the equation for predicted load, we obtain a formula that predicts the optimal load based on body length:

$$M^* = \frac{(0.0004755 \bullet (4.8854 \bullet L - 11.2126) + 0.02912)}{0.0009510}$$

$$M^* = 2.4427 \bullet L + 25.02$$

Briefly, if we recall that the foraging rate equation was a concave down parabola that passed through the origin, we can easily see that this optimal load will always be half the load that the ant would need to carry to reduce its speed to zero. Since velocity and load are linearly related for a given ant body length, this also means the predicted velocity associated with the optimal load is half the velocity we would predict the ant to travel without any load.

PECCARY DISTURBANCE AND IMPACT ON PLANT COMMUNITIES OF LA SELVA TROPICAL FOREST

ANNA L. DEFFEBACH, SHEA E. FLANAGAN, AND JAMIE E. MCCLAUGHLIN

Faculty Editor: Matthew P. Ayres

Abstract: Conservation efforts to preserve land may sometimes produce increases of large omnivore populations that lack top-down controls. These omnivores may affect the ecosystem in unexpected ways. At La Selva Biological Station, the white-collared peccary (*Pecari tajacu*) population has dramatically increased near the station in recent years, from 2002-2012. We measured two peccary food sources, walking palms (*Socratea exorrhiza*) and almendro trees (*Dipteryx panamensis*), at sites near and far from the station. We measured age class and percent of roots touching the ground for walking palms and percent of seeds eaten for almendro trees. We found far more immature palms at the far site than the near site, and no recruits at the near site. We also observed a higher percent of almendro seeds eaten at near sites than far sites. It seems that peccaries are already impacting the environment, and there is reason to expect continued impacts that affect multiple species.

Key Words: almendro, walking palm, extinction threshold, *Pecari tajacu*, *Dipteryx panamensis*, *Socratea exorrhiza*

INTRODUCTION

Conservation efforts around the world are working to counteract human environmental impacts. Setting aside areas of protected land is a popular and effective solution to the ever-increasing stress of habitat loss. However, as we isolate these conservation areas with the aim of preserving wildlife, organisms and ecosystems respond in unintended ways. A potential response, observed in many conservation areas, is an increase of large omnivore populations (Terborgh et al. 2001).

Possible causes for these increasing omnivore populations are the losses of both top-down and bottom-up controls. Historically, large predators have been disproportionately impacted by hunting and human-induced habitat fragmentation. As large predators require more time and larger tracts of land to rebound to their original population size, their numbers remain too low to limit the more tolerant omnivores. Along with this loss of natural top-down control, eliminating human hunting pressures in a park also releases

the large herbivore populations. Furthermore, as land is conserved the bottom-up controls of limited food and habitat availability are decreased. This combination of these factors could explain why omnivore populations are increasing in many conservation areas around the world.

Despite these changes, it remains less clear how an increase in omnivore populations will have an effect on the community that surrounds them. In general, large omnivores have the potential to cause a great deal of physical disturbance by trampling, wallowing, rooting around and causing local erosion. An increased omnivore population may also deplete their food source thereby shifting the type and abundance of local primary producers toward less palatable-plants. In particular, omnivores that eat plant parts necessary for growth and asexual reproduction such as seeds and roots may have large demographic impacts.

However, the impact of this increase in the population of one trophic level may not necessarily lead to meaningful impacts on a

community, depending on the areas' ecological resilience. For example, a small-scale change in native fauna may not affect large, complex ecosystems such as tropical rainforest. Other factors may contribute to stabilizing density-dependence on the populations negatively influenced by the herbivores. As the population of a food source species decreases, the remaining individuals of that species may eventually experience less predation from herbivores as their abundance becomes too low to be sought after as a food resource. The role of redundancy that the plant species plays in the ecosystem may be another source of ecosystem resilience. If a certain amount of ecological redundancy exists, the community may replace the resource with similar species, minimizing the effect of species loss on the environment.

To determine whether an increased omnivore population is affecting an ecosystem, known food sources could be surveyed for evidence of herbivory. We hypothesized that if the omnivores were having an effect on the ecosystem, herbivory damage would be greatest in areas of high omnivore density.

Study Site

La Selva Biological Station, located in northern Costa Rica, is a 1,600 hectare area set aside in 1954 for the purpose of protecting fauna and conducting biological study. Since around 2002 residents of La Selva have begun to note an increased abundance of *Pecari tajacu* (white-collared peccary) near the research station (Orland Vargas and Danelo Brenes, *pers. comm.*). This apparent increase in *P. tajacu* populations is suspected to have negative impacts on the local environment but little has been done to quantify the effects of the increased peccary population.

To determine whether the increased *P. tajacu* population is effecting the environ-

ment, we measured evidence of herbivory on two of the peccaries' known food sources: *Socratae exorrhiza* (walking palms) and *Dipteryx panamensis* (almendro trees). *S. exorrhiza* have aerial, adventitious roots, which function as support as well as for nutrient acquisition (Lieberman and Lieberman 1987). Herbivory on these roots may cause palms to lack adequate nutrients and to lose structural stability. Peccaries are also seed predators of *D. panamensis*. By eating and killing seeds, peccaries have the potential to decrease *D. panamensis* populations.

We chose *S. exorrhiza* and *D. panamensis* as measures of peccary herbivory because while many different organisms may feed on walking palm roots and almendro seeds, peccaries were the only animals that we observed feeding on the almendro seeds on the ground. An animal needs extremely powerful jaws to break the hard almendro seeds open, which peccaries can do. Walking palms can drop their roots for reasons other than herbivory. We observed especially high herbivory on palm roots in peccary wallowing and resting areas, which matched reports of local naturalists that peccaries eat the roots of walking palms.

METHODS

On 13 and 14 February 2012, we set up 10 transects each in near and far locations relative to the biological station at La Selva Reserve, Costa Rica. Near locations ranged from approximately 150 m to 250 m from the station, and far locations were approximately 7000 m from the station. Walking along each 20 m transect 6 m wide, we recorded the number of walking palms (*S. exorrhiza*), their age class, number of total stilt roots, and number of stilt roots reaching the ground. Each transect was separated from the next by

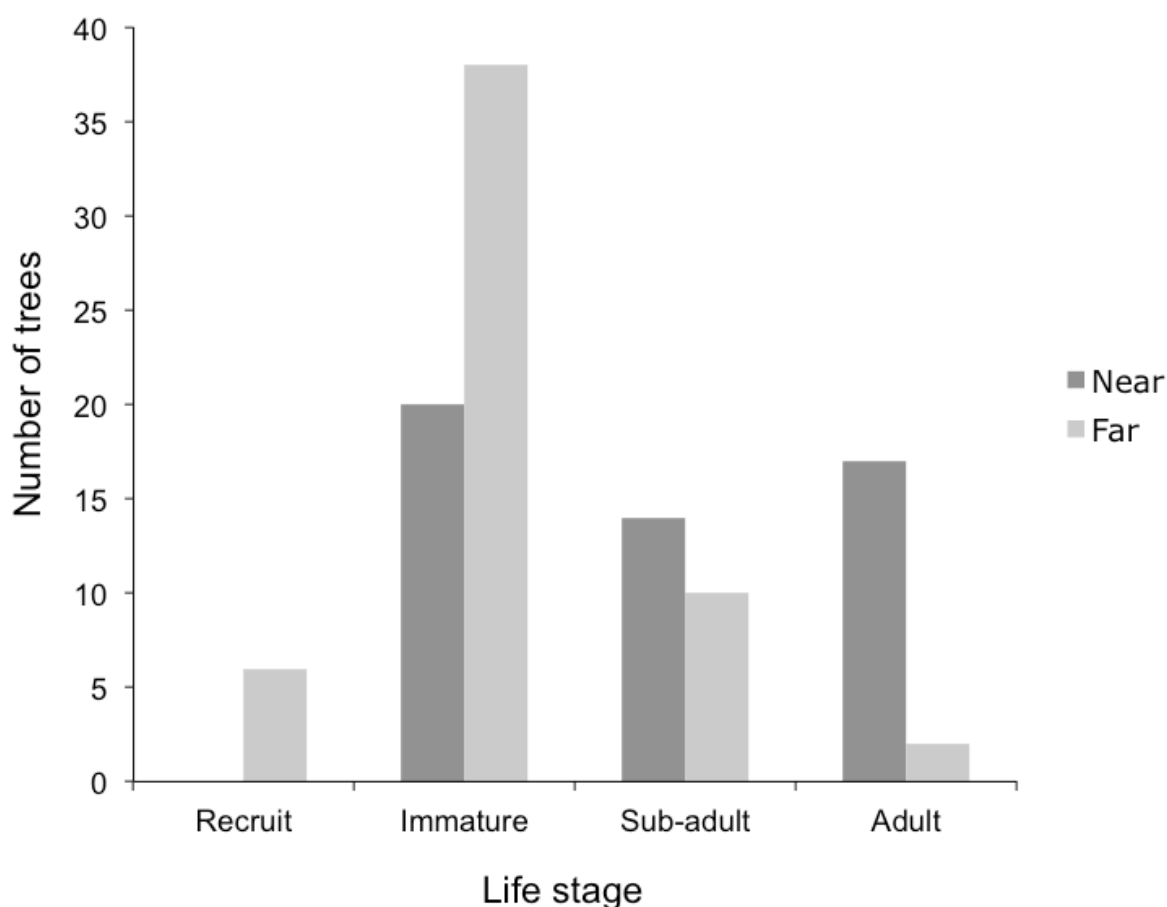


Fig. 1. The distribution of *S. exorrhiza* life stages differed between near and far study sites.

10 m, and we alternated which side of the trail we placed the transect.

On 15 February 2012, we counted almendro seeds from *D. panamensis* trees in 1 by 1 m plots in secondary forest as a gradient of peccary grazing from near to far locations relative to the biological station. We sampled three plots per tree for 6 almendro trees, counting the number of whole seeds and eaten seeds in each plot. An eaten seed was defined as a seed that had been cleaved in half (apparently by a peccary).

We used a chi-square test to determine if there was a difference in the distribution of age classes between the near site and the far site. We performed a nested variance analysis

to determine if walking palm trees were auto-correlated within transects. We found that the variance among transects was not a significant factor, allowing us to proceed with a two-tailed t-test of *S. exorrhiza* palms as individuals (Table 1). The nested ANOVA also indicated that seed predation varied among trees, limiting our scope of statistical inference concerning almendro seed predation. All analyses were performed using JMP 9.0 software (SAS Institute, Cary, NC).

RESULTS

There was a significant difference in distribution of *S. exorrhiza* life stages between

near and far study sites ($X^2 = 18.09$, $df = 2$, $P < 0.001$; Fig. 1). Trees in the near study site had a significantly lower percent of roots reaching the ground (ANOVA, $F_{1,16} = 21.95$, $P < 0.0001$; Fig. 2; Table 1). The mean percent of eaten *D. panamensis* seeds for trees from the far site was 84 ± 6 (mean ± 1 SE) while the mean percent eaten was 50 ± 15 (mean ± 1 SE) for the near site. All trees at the near study site had a greater percentage of seeds eaten than any tree at the far study site, but there were only 5 trees in total and the effect was not statistically significant (ANOVA, $F_{1,4} = 4.11$, $P = 0.11$; Table 2).

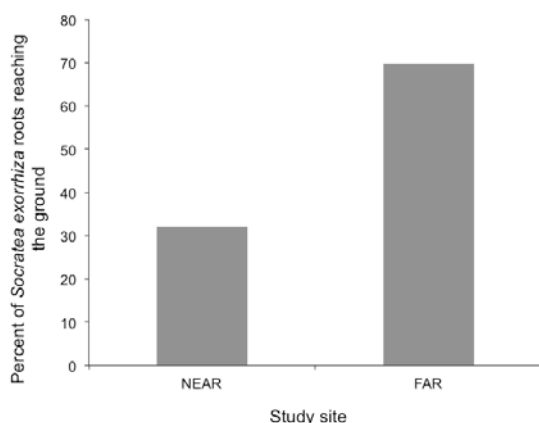


Fig. 2. *S. exorrhiza* trees at the near study site had a significantly lower percent of roots reaching the ground.

DISCUSSION

Table 1. A nested variance analysis showed variance among *S. exorrhiza* between locations was significant and variance between transects within a location was not a significant factor.

Source	DF	MS	F	Prob > F
Location	16	0.047	0.56	0.91
Transect[Location]	1	1.38	21.95	<0.0001

Our results suggested that walking palms were noticeably impacted by peccary herbivory. The young palm populations were different between sites. There were no recruit palms in the near site and only half as many immature palms compared to the far site. If

there is really no recruitment of palms, the local population will go extinct, since walking palms cannot reproduce asexually.

Additionally, fewer walking palm aerial roots reached the ground at the near site compared to the far site, suggesting a difference in herbivory. As this gradient in damage corresponded to the observed gradient of increased peccary density near the station, it appeared that this difference was at least partly attributed to peccary herbivory. Furthermore, since walking palms depend on their roots for stability as well as nutrient uptake, herbivory on roots could cause older palms to fall over or die from nutrient depletion.

Peccaries may also be impacting almendro tree populations. The difference in percentage of *D. panamensis* seeds eaten between the two sites was large enough to be important if real. Thus, peccaries could have long-term effects on almendro tree population, to the extent that seeds are limiting to the seedling recruitment. Stronger inferences regarding almendro trees will require more data.

If almendro trees become less populous due to peccary seed predation, these consequences may extend more broadly. For instance, the endangered great green macaw, which depends on almendro seeds as a food source, could shift even closer toward their extinction threshold (BirdLife 2008). Furthermore, this could result in a destabilizing feedback as the macaws are seed dispersers, so depressing their population would further limit the abundance of almendro trees.

Table 2. A nested variance analysis showed variance among *D. panamensis* tree plots within a location to be greater than variance between plots.

Source	DF	MS	F	Prob > F
Location	1	0.50	4.11	0.11
Tree[Location]	4	0.12	6.01	0.007
Error	12	0.02	N/A	N/A

Since both almendro trees and walking palms require fertilization to produce offspring, another plant of the opposite sex must be within pollination distance. Therefore, as peccary herbivory potentially decreases the density of almendro trees and walking palms in the near future, it could become more difficult for the plant populations to reproduce. The effects of peccaries on walking palms is more noticeable than that on almendro trees because walking palms are faster-growing than almendro trees, therefore it may take a longer period of time to see the effects on the almendro tree population.

Overall, the loss of top-down control on large omnivore populations, as seen in the peccary population in La Selva, may ultimately affect many elements of the surrounding ecosystem. Therefore, conservation efforts must attempt to foresee and control these challenges when preserving the earth's wildlife.

ACKNOWLEDGEMENTS

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THE EFFECTS OF FOREST STAGE ON AVIAN ACOUSTIC COMMUNICATION

WALES A. CARTER

Faculty Editor: Matthew P. Ayres

Acoustic communication is used in birds for species recognition, territorial displays, mate selection, and kin recognition. The importance of these processes creates a high selective pressure to optimize the efficacy of communication. The Acoustic Adaptation Hypothesis (AAH) suggests that species will adapt to optimize the transmission of acoustic signals based on the structure of their habitat, with denser vegetation reducing the effectiveness of calls at high frequencies and with complex subunits. I tested the effects of forest type on bird calls at La Selva Biological Station, Costa Rica, to assess the importance of acoustic structure on avian communication. I found that bird calls in dense primary forests were characterized by lower frequencies, and complexity based on call length, number of subunits, and range of frequencies.

Key words: Acoustic ecology, Acoustic Adaptation Hypothesis, avian communication, primary forest, La Selva

INTRODUCTION

Communication is widely employed by animals for a wide variety of functions that include species recognition, territorial displays and defense, mate selection, and kin recognition (Searcy and Anderssen 1986). The importance of these factors thus in turn provides selective pressure to optimize the efficacy of communication (Mathevon, et al. 2008). For many birds and other widely dispersed animals the need for rapid communication limits the primary means of communication to those able to cover large distances with speed: visual and acoustic communication. In dense forests, which are common in the tropics, visual communication is limited by the dense vegetation, leaving acoustic communication as the primary method of transmission in many species. This still presents problems as the same dense vegetation is capable of both degrading and altering vocalizations (Nemeth et al. 2001). Calls may experience decreases in amplitude, frequency filtration, and a decrease in signal to noise ratio as a result of interference by vegetation (Nemeth et al. 2001). These observations have led to the creation of the

Acoustic Adaptation Hypothesis (AAH), which posits that calls at low frequencies and low subunit complexity are more resistant to degradation and should be selected for in areas with dense vegetation (Boncoraglio and Saino 2007). Over time, calls are expected to be adapted to maximize the distance over which they are effective by reducing frequency, frequency range, and subunit complexity.

In the tropical lowland forests of Central America, there has been an increasing trend for primary forest to be converted to other uses, primarily agriculture. Even when the forest regenerates, a process that can take decades, it takes a considerable amount of time to regain the original structure and density of uncut forest. If bird species are indeed adapted to optimize the transmission of their calls in a particular type of forest structure, these changes in forest type may have a considerable effect on their ability to communicate. I investigated the effect of forest type on bird calls in an effort to determine the importance of forest structure to avian communication in a tropical lowland forest. I tested the hypothesis that bird calls would be at a lower frequency and have a

lower subunit complexity in the denser primary forest, as predicted by the AAH.

METHODS

I examined the effect of forest type on the frequency and complexity of bird calls from 12-14 February 2012 in two plots at La Selva Biological Station, Costa Rica. I haphazardly selected one plot in primary forest, and one in secondary forest. I characterized the forest at each plot by measuring the canopy density (measured with a spherical densitometer) and maximum distance at which a flag of surveyor's tape at eye level was visible in each cardinal direction at 4 sites at least 50 m from each plot. I then recorded bird calls at each site between 0730 and 1800 for 3 days with a VN-6200PC digital voice recorder (Olympus Imaging Corp., Tokyo Japan) set on variable control voice actuator (VCVA) with a sensitivity of 12. This setting only recorded in the presence of sounds that achieved a reasonably low amplitude threshold. On the fourth day, I placed both recorders 500 m apart in the secondary growth forest to achieve a more even sample size. I isolated and processed individual calls

using Ravenlite 1.0 interactive sound analysis software (Cornell Lab of Ornithology, Ithaca NY). I only selected calls that were entirely distinguishable from the background noise. To allow for some intraspecific variation, I accepted up to 5 songs from a given species, each separated by at least 5 minutes. I then extracted the frequency of the call at its maximum amplitude, the range of frequencies covered by the call, the length of the call, the number of subunits in the call, and the mean subunit length from the spectrograph of each call (Fig. 1). I compared the canopy density, visible distance, and frequency at peak amplitude between forest types with two-sample *t*-tests. To combine range, length, subunits, and subunit length into measures of complexity, I performed a Principal Component Analysis (PCA), which produced two variables, PC1 and PC2. I then tested the effect of forest type on each of these variables with two-sample *t*-tests. I performed all statistical analyses using JMP 9 software (SAS Institute, Cary NC).

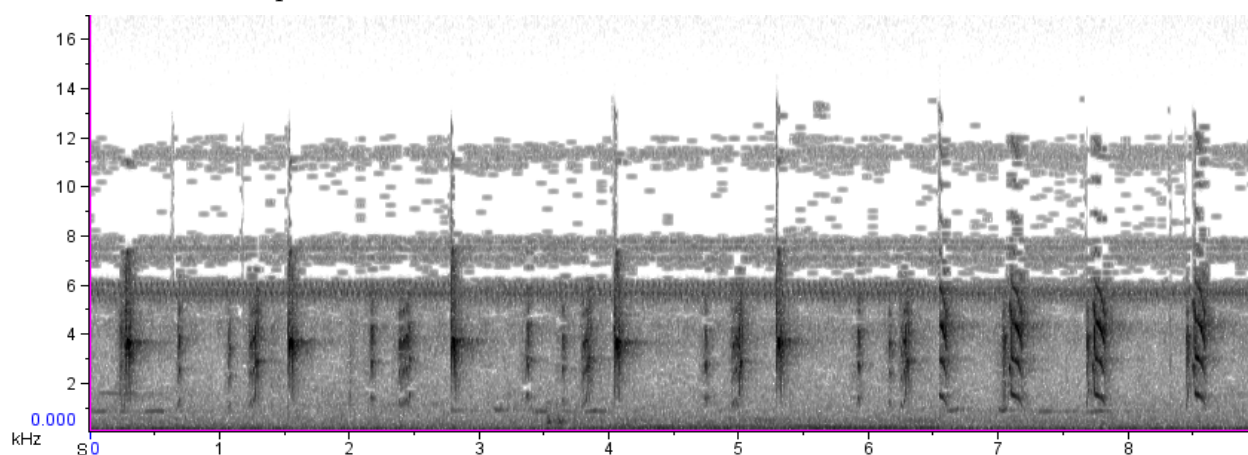


Figure 1. A sample spectrograph of a bird song recorded in the primary neotropical rainforest of La Selva, Costa Rica.

RESULTS

On average, the canopy of my secondary forest plot contained 10.5% open space, significantly more than the 6.4% percent open space in the primary forest canopy ($t_{21.5} = 3.95$, $P < 0.001$; Fig. 2). Likewise, the understory of the secondary forest was more open than that of the primary forest. Mean distance from which a surveyor's tape flag could be seen was 11.6 m versus 8.6 m ($t_{30} = 2.29$, $P = 0.015$; Fig. 3).

There were fewer bird calls lower in the secondary forest than in the primary forest. In total I processed 20 distinctly different calls from the secondary forest and 17 from the primary forest. I could not identify most call to species, but it appeared that there was only one species, the Chestnut-Mandibled Toucan, that was recorded in both forest types. Bird calls in the secondary forest had a mean frequency of 3849 Hz, whereas calls in the primary forest had a mean frequency of 2656 Hz ($t_{52} = 2.83$, $P = 0.006$).

Frequency range, call length, number of subunits, and mean subunit length were all positively correlated (Table 1). The first axis of a principal components analysis (PC1) had positive loadings from frequency range, call length, and number of subunits, while PC2 increased with a greater subunit length and a smaller number of subunits (Table 2). PC1 was significantly greater in the primary

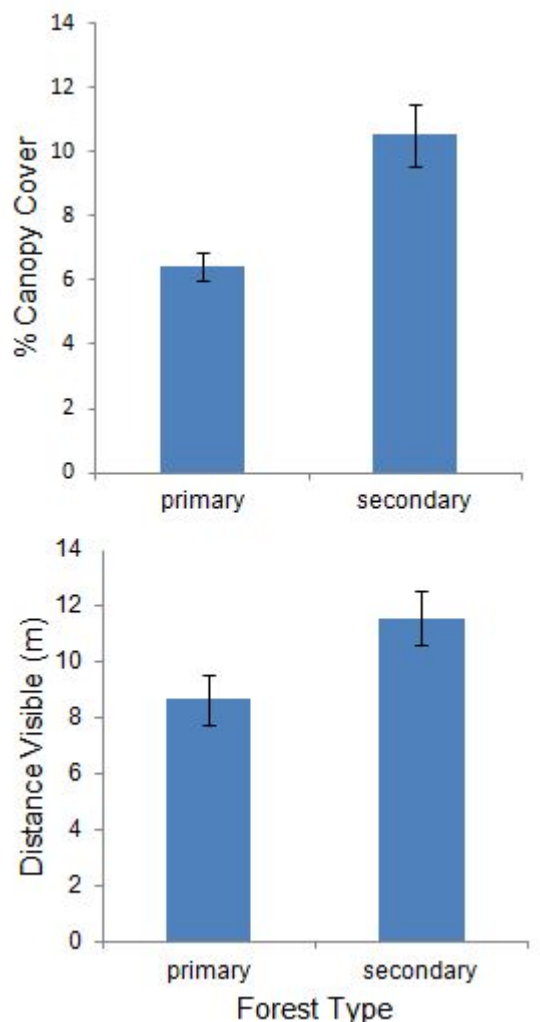


Figure 2. Percent canopy cover (upper) and distance that a surveyor's tape flag at eye level was visible (lower) in primary vs. secondary forest at La Selva Biological Station, Costa Rica.

forest than the secondary forest ($t_{39.8} = -2.86$, $P = 0.007$; Fig. 4), but there was no significant difference in PC2 between forest types, although it was slightly larger in the secondary forest than the primary forest.

Table 1. Correlation matrix of variables used in Principal Components analysis of bird call complexity.

	Freq. Range	Length	# subunits	Subunit length
Freq. Range	1			
Length	0.47	1		
# Subunits	0.28	0.80	1	
Subunit Length	0.33	0.42	0.01	1

Table 2. Loading scores from a Principal Components Analysis of bird calls

Variable	PC1	PC2
Freq. Range	0.45	0.31
Length	0.63	-0.15
# Subunits	0.52	-0.58
Subunit length	0.35	0.74
percent	56 %	26%

DISCUSSION

The difference in both canopy and understory density between primary forest and secondary forest presents a clear dichotomy of structural habitats which birds can select from. Such a difference would need to be present for there to be differential selection on characteristics of bird calls as is predicted by the AAH. This supports my assumptions of the effects of forest regeneration on forest, and allowed me to test the effects of forest type on avian acoustics.

I also found that birds used higher frequency calls in secondary forest than in primary forest. This finding supports the predictions of the AAH. Considering that sound is known to degrade with interference by vegetation, it seems that a possible explanation of this difference is a response to the different structural environments of each forest type. High frequencies are more susceptible to deflection or absorption by vegetation, and so there is likely to be selection against that characteristic in primary forest, resulting in the pattern I observed. Any selection on these characteristics would require time to accrue, in all likelihood more than has existed in this site. This could be explained by the adaptation of spatially isolated bird populations to different acoustic structures accompanied by a trait leading to the selection of habitats with specific characteristics. After habitats were disturbed, they would then be more likely to be recolonized by those species adapted to the acoustic structure of the regenerating area. In La Selva, the secondary forest may have been colonized by birds adapted to different acoustic environments.

Complexity can be described in several ways which may confer different

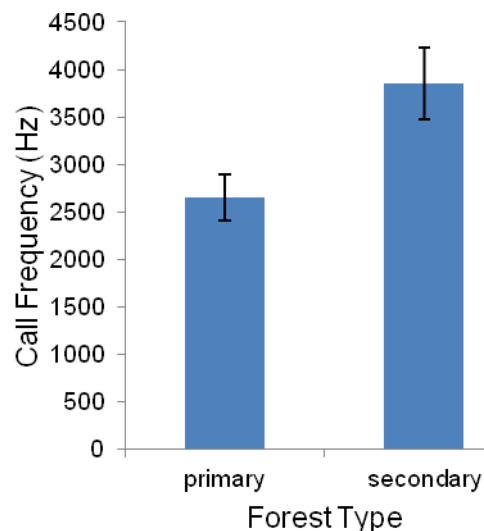


Figure 3. Mean bird call frequency in primary vs. secondary forest at La Selva Biological Station, Costa Rica.

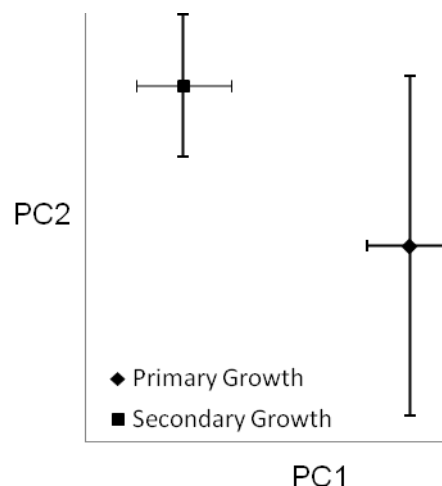


Figure 4. Comparison of bird calls recorded in primary vs. secondary forest. PC1 and PC2 were extracted from measurements of four parameters (Tables 1-2) by principal components analysis. PC1 increased with the range of frequencies, the length of calls, and the number of subunits. PC2 increased with a greater subunit length and a smaller number of subunits.

advantages in different acoustic environments while still allowing for the transmission of large amounts of information. For example, individual subunits of songs may be long and cover a wide range of frequencies, although these qualities make the call more susceptible to degradation.

Alternately, the entire call may be long and contain a large number of short subunits in a narrow range of frequencies, which makes it more robust in dense vegetation (Irwin 2000). In my analyses, PC2 increases with a greater subunit length and smaller number of subunits, both characteristics of the former description of complexity. Thus, according to the AAH, PC2 would be expected to be greater in less dense habitats. This was the trend in my data, but the differences were not significant, possibly indicating that the difference between habitats was not distinct enough to provide a distinct structure to drive differential habitat selection. PC1, however, was composed from increased frequency range, length, and number of subunits, which is more similar to the second measure of complexity. The AAH predicts that the range of frequencies should decrease in habitats with dense vegetation, so the combination of these factors to increase the overall complexity of a call is counter to the expectations derived from the AAH. This suggests that other selective pressures are also responsible for the evolution of bird calls to their present form and the selection of habitat that results.

The AAH provides a general model for explaining acoustic communication based on habitat. It is broadly applicable because of its focus on the physics of sound transmission, but it is also handicapped by the assumption that distance of transmission should be maximized. Other selective pressures, notably interception by predators and parasites, could very easily alter the selective pressures of forest structure. It is also possible that there are adaptations to extract information from degraded calls rather than to change the calls themselves. Thus, it may be reasonable to view the acoustic structure of a forest as the constraint under which these species have

evolved. Other factors may provide stronger selective pressures at this point which confound its effects and result in much of the variation observed in nature.

Nevertheless, there is considerable evidence that acoustics have shaped the way birds communicate. Knowledge of the factors affecting sound transmission is essential to understanding how birds are adapted to their acoustic environment, how this affects species composition in a given area, and how they will respond to changes that environment. Considering the rate of environmental change at the present, such an understanding will likely grow in importance for conservation and recolonization efforts in the future.

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SPLASH OF THE CHITONS: DISTRIBUTION AND MOVEMENT OF *ACANTHOPLEURA GRANULATA* IN THE ROCKY INTERTIDAL ZONE

WALES CARTER, MILO JOHNSON, BENJAMIN KESSLER AND JAMIE McLAUGHLIN

FACULTY EDITOR: CELIA Y. CHEN

Abstract: Intertidal zones are harsh environments in which organisms display a suite of unique adaptations in both physiology and behavior. Fuzzy chitons, *Acanthopleura granulata*, mollusks of the class Polyplacophora, have existed for over 400 million years and are common along the shorelines of Little Cayman. We measured tide pool characteristics, chiton abundance, dispersion within pools, and movement (diel and nocturnal) to describe distribution and movement patterns that may function as adaptations to tide pool environments. Chitons were aggregated only in pools that experienced constant wave action, perhaps as a mechanism to avoid desiccation. Within pools, chitons were clumped, suggesting the selection of similar suitable microhabitats by individual *A. granulata*. Contrary to our expectations, chitons did not display any cyclical diurnal or nocturnal movement patterns.

Keywords: Abiotic stressors, *Acanthopleura granulata*, booster shot, chiton, dispersion, tide pool

INTRODUCTION

Organisms inhabiting rocky intertidal zones are subjected to a relentless cycle of harsh conditions coming from both water and air. Competition and predation from marine species reign in the water below while extreme temperatures and the threat of desiccation loom above (Garrity 1984). Tidal fluctuations and diel temperature changes drastically change the importance of these effects, constraining the vertical distribution of animals within intertidal zones (Begon 1996, Schill *et al.* 2002). To cope with daily shifts in environmental conditions, intertidal species have evolved an array of behavioral adaptations (Garrity 1984). For example, gastropod species living on a tropical rocky shore exhibit aggregation tendencies, foraging patterns, and refuge-seeking behavior related to diel-tidal fluctuations (Garrity 1984, Stafford and Davies 2004).

Chitons, mollusks of the class Polyplacophora, are common littoral species along Caribbean shorelines that generally live near the ocean and graze the rocky substrate

for microflora (Glynn 1970). Though chitons appear to be well protected by their thick shell and strong attachment to the rock, they face predation from fish and other marine organisms. Heightened predation tends to occur at high tide when marine predators have greater access to tide pools (Focardi and Chellazi 1990). Chitons must also contend with the risk of desiccation, especially from the intense solar radiation of the tropics (Harper and Williams 2001).

The fuzzy chiton, *Acanthopleura granulata*, is abundant along the rocky intertidal zones of Little Cayman Island. It is unclear what adaptations, behavioral or otherwise, *A. granulata* employs to cope with these harsh environments. We investigated characteristics of *A. granulata* distribution that may be adaptations for life in the rocky intertidal zone. We examined components of their habitat selection, aggregation behavior, and their diel-tidal movements. Based on studies of intertidal gastropods, we hypothesized that aggregation and vertical movement patterns would vary with diel-tidal changes, in response to associated

changes in the likelihood of predation or desiccation (Garrity 1984, Levings and Garrity 1983).

METHODS

We investigated the abundance, distribution, and movement of *Acanthopleura granulata* on 24-28 February 2012 along a 70 m stretch of the rocky intertidal zone in Spot Bay on the north shore of Little Cayman. We measured tide pool circumference, tide pool depth from rim to bottom, wave action, and chiton abundance in 15 haphazardly selected pools. We only selected pools with chitons present. We used the circumference to calculate the area of each pool based on the approximation that pools were circular. We then used this area to calculate the chiton density in each pool. We measured wave activity as the total number of waves strong enough to wash the entire area of the pool during a 2 minute period.

To determine the spatial distribution of *A. granulata*, we measured the distance between each chiton and its three nearest neighbors in four pools (N=106) at 1500 on 26 February at low tide. We also noted whether each chiton was submerged, on the edge or out of the water and measured the width of each chiton's fourth valve as a proxy for age (Fathey 1996). To investigate the changes in spatial distribution of *A. granulata* over time, we repeated these measurements in the first two pools again at 0100 and 1000 on 27 February, both times at high tide.

We also tracked the vertical movement of *A. granulata* by marking 15 individuals and their position on the pool wall with a paint marker at 1000 on 26 February 2012 in each of three pools (N = 45). We then measured the difference between each chiton's current position and original position at 5 subsequent

time intervals: 1630 on 26 February, 0100, 1000, and 1630 on 27 February, and 0930 on 28 February. Due to intense wave action in one pool and difficulties with marking, some periods had lower sample sizes (N=24-41).

Statistical Analyses

We tested the relationship between wave action and chiton density using a linear regression. To test the effect of pool depth on chiton density we used a linear regression.

To examine the spatial distribution of *A. granulata*, we first calculated the mean population density within the four pools. We then used equation 1 to calculate an expected mean nearest neighbor distance assuming a random distribution, where λ is the mean population density (Clark and Evans 1954), and compared this expected value to the actual mean nearest neighbor distance.

$$E(r) = \frac{\sqrt{1/\lambda}}{2} \quad (\text{Equation 1})$$

We then compared this expected mean nearest neighbor distance with our observations of nearest neighbor distance using a Wilcoxon Signed-Rank test. To determine whether aggregation varied with size we used a linear regression to test the relationship between chiton width and the average distance of its three nearest neighbors. To determine whether aggregation might be a strategy to avoid predation and/or desiccation, we used a one-way ANOVA to compare the mean distance to the three nearest neighbors between chitons above, below, and at the pool waterline. We compared the effect of time of day on average neighbor distance with a one-way ANOVA. We tested the effect of tide on average neighbor distance with a two-sample *t*-test.

RESULTS

We observed chitons only in pools with wave action, all of which were one meter or less above sea level at low tide. We found a significant positive relationship between wave action and chiton density at low tide (linear regression; $R^2 = 0.40$, $F_{1,10} = 6.06$, $P = 0.036$; Figure 1). However, when we included the four pools surveyed at high tide, we found no significant relationship between wave action and chiton density. Additionally, there was no effect of pool depth on chiton density.

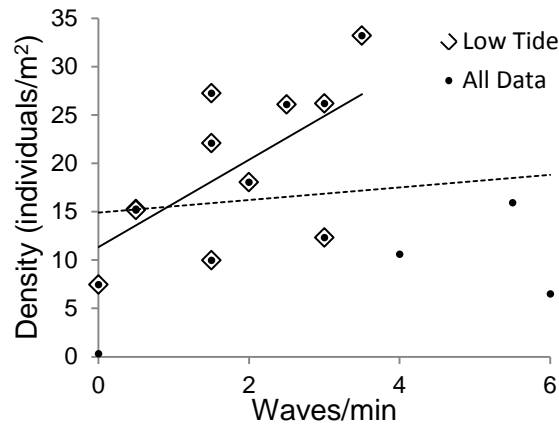


Fig 1. *Acanthopleura granulata* density increased with greater wave action. This trend was significant during low tide ($R^2 = 0.40$, $F_{1,9} = 6.06$, $P = 0.036$), but not when the 4 pools measured at high tide were included.

We found that the nearest neighbor distance of *A. granulata*, 6.9 cm, was significantly lower than our expected distance of 12.5 cm (Wilcoxon Signed-Rank test; $S_{105} = -2189$, $P < 0.001$). Also, there was a trend towards a smaller mean distance to the three nearest neighbors at 1000 than at 1630 or 0100. There was no significant correlation between chiton width and mean distance to the three nearest neighbors. Likewise, there was no significant effect of relative water position or

tide on mean distance to the three nearest neighbors.

We found that *A. granulata* moved consistently downwards over the course of our observations, a pattern that does not correlate with tide or diurnal cycles (Figure 2).

DISCUSSION

Acanthopleura granulata was more abundant in pools with frequent wave action, possibly as an adaptation to prevent desiccation and allow cooling while avoiding the high temperatures and salinities of stagnant pools. The occupation of the splash zone by chitons could also be a response to the threat of predation, as they are out of the range of marine predators. Even for those chitons that are submerged, frequent wave action may make their pools inconvenient hunting grounds for marine predators.

We also found that *A. granulata* display aggregated dispersion. This pattern did not change with the level of the tide and only exhibited a slight change with the time of day. Though aggregative behavior has been demonstrated in some gastropods as a mechanism to prevent desiccation (Garrity 1984), we do not believe this to be the case for *A. granulata*. If *A. granulata* were aggregating to preserve moisture we would likely have seen much closer aggregation during the day, especially during the afternoon low tide. Aggregative behavior also does not appear to be a response to predation. We observed several relatively solitary chitons persisting over days, a phenomenon that would be unlikely if such chitons were quickly identified and consumed by predators. We suggest that the aggregated dispersion of *A. granulata* is due to microhabitat selection based on a combination of substrate, wave

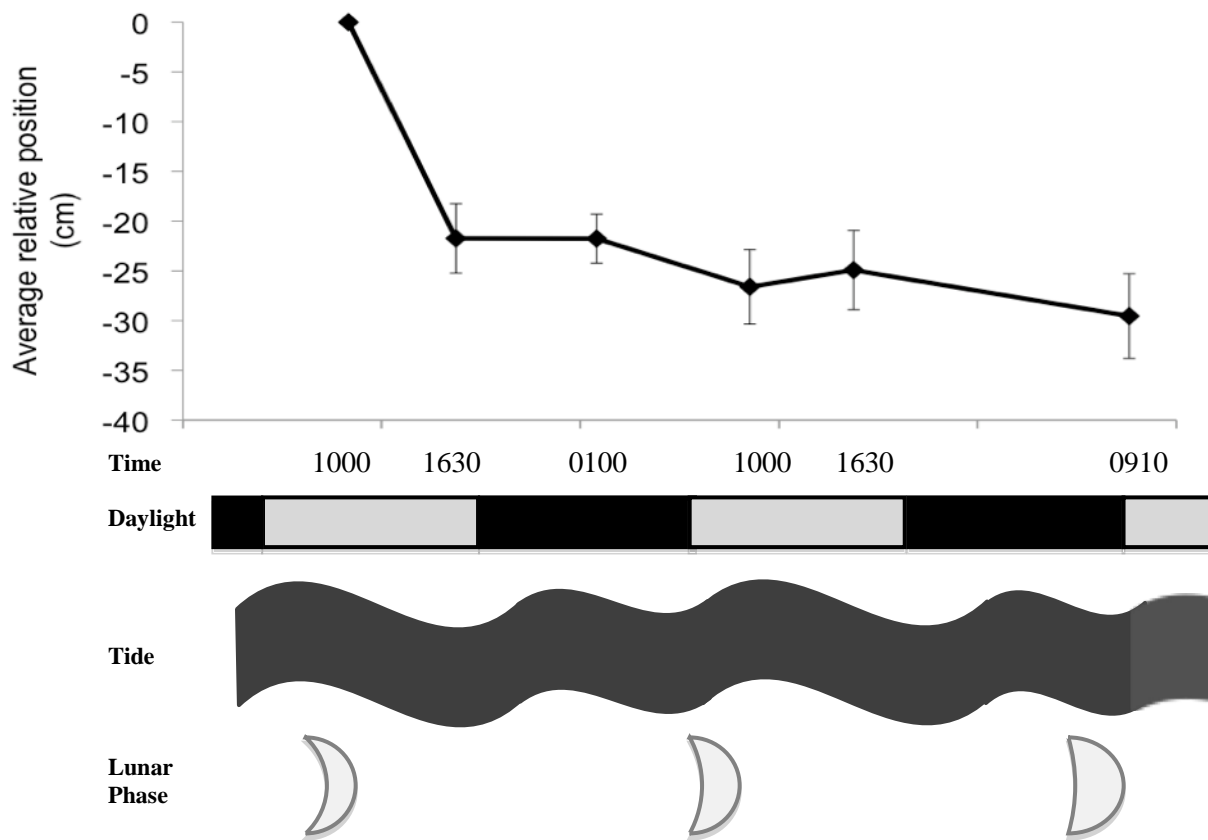


Fig 2. *Acanthopleura granulata* movement patterns over 48 hours (N=24-41). Error bars are one standard error. Data was collected at the Central Caribbean Marine Institute, Little Cayman.

action, shade, shelter, and food resources. We expected *A. granulata* to move vertically with the tidal cycle in order to stay in an optimal habitat. We did not find this pattern, nor did we find any cyclical movement patterns between day and night. Rather, *A. granulata* continually moved downward, never returning to the height at which we observed them the first afternoon. It is unclear what mechanism is responsible for this phenomenon, although it is possible that it is associated with the lunar cycle. The shift towards neap tide may play a role in the consistent descent of these chitons, as the vertical upper limit of the chitons' range could be determined by the high tide. Additionally, as *A. granulata* spawn in rhythm

with the new and full moon (Glynn 1970), their movement could be linked to lunartropic mating behavior. Further studies are needed to investigate these potential effects.

Chitons have existed on this Earth for over 450 million years (Runnegar 1974). Like other intertidal invertebrates, they must have physical and behavioral adaptations to survive in their harsh environment (Garrity 1984). Therefore, while we did not observe any specific diel-tidal behavioral patterns, *A. granulata* clearly has a successful life history strategy. Through eons of changing environmental pressures, these primitive mollusks have persisted using their simple

and ancient adaptations.

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A NOTE ON THE EPIDEMIOLOGY OF DARK SPOT DISEASE ON LITTLE CAYMAN ISLAND

ROBIN A. COSTELLO, ANNA L. DEFFEBACH, MADILYN M. GAMBLE, MADELINE K. KREHER, JESSE T. RIEB, AND AMY E. VAN SCOYOC

FACULTY EDITOR: CELIA Y. CHEN

Abstract: Coral diseases, one of many factors contributing to a worldwide decline of coral reefs, have increased in prevalence and virulence over the last few decades. However, there is still very little known about what causes these diseases, how they are spread, and what factors might make certain coral colonies more susceptible than others. In this study, we investigated the spatial distribution as a proxy for transmission of Dark Spot Disease in massive starlet coral (*Siderastrea siderea*). We sampled coral colonies for diseases at two sites on Little Cayman Island, and used spatial analysis to look for large-scale differences (evidence of dispersal by ocean currents) and small-scale clumping (evidence of transmission through biological vectors such as fish or invertebrates). Since Dark Spot Disease spreads at a constant rate, we used area of disease coral tissue on each colony as a proxy for time since infection. We also measured two stress indicators, hyperplasm and encroachment of algae, to see if they correlate with disease. We found no correlation between stress indicators and disease, no significant spatial clustering of diseased or healthy corals, and no difference in the percent of corals infected between our two sites. However, the mean size of disease patches on individual colonies was greater at the northeast site than at the southwest site, suggesting that this site has been diseased for a longer period of time. This is consistent with the pattern that would be expected if the disease were transmitted via the prevailing east-west current. The lack of spatial clustering within each site does not support the biological vector hypothesis. This random distribution of disease within each site also suggests the possibility of variation in the susceptibility of coral colonies to the disease.

Key Words: epidemiology, dark spot disease, spatial analysis, massive starlet coral (*Siderastrea siderea*), stress response.

INTRODUCTION

Coral populations are dramatically declining worldwide due to increases in storms, ocean temperature, overfishing, sedimentation, agricultural runoff, and coral diseases (Raymundo et al. 2009, Richardson 1998). Recently there have been notable increases in the prevalence and virulence of coral diseases, particularly in the Caribbean (Richardson 1998, Nugues et al. 2004). Fifteen new coral diseases were recorded in the 1990s alone (Richardson 1998). Despite this alarming increase in disease and its implications for coral populations, the etiology and epidemiology of many coral diseases are still shrouded in mystery (Jolles et al. 2002).

Only four coral diseases (black band,

white plague type II, white band, and Aspergillosis) have resulted in documented coral mortality via confirmed pathogens (Richardson 1998). Studies on these pathogenic diseases have generated three different hypothetical mechanisms for disease dispersal: transmission of pathogens through water currents (Jolles et al. 2002); transmission by vectors such as snails, fireworms, butterfly fish, or macroalgae (Williams and Miller 2005, Nugues et al. 2004, Raymundo et al. 2009); or, in the case of branching and soft corals, transmission through direct contact between two conspecific colonies (Williams and Miller 2005, Jolles et al. 2002). Not all mechanisms of disease dispersal are relevant for all coral diseases and coral species.

Dark Spot Disease (DSD), also known as

dark spot syndrome, was first described in the early 1990s off the coast of Colombia and has since spread throughout the Caribbean (Gil-Agudelo et al. 2004). DSD affects three dominant scleractinian coral species, including Boulder Star Coral (*Montastrea annularis*), Blushing Star Coral (*Stephanocoenia intersepta*), and Massive Starlet Coral (*Siderastrea siderea*), (Gil-Agudelo et al. 2004, Humann and Deloach 2002). While DSD spreads slowly on infected colonies, understanding DSD transmission is essential due to its wide geographic range and high incidence on dominant reef-building corals (Gil-Agudelo et al. 2004). DSD results in tissue death on approximately half of affected colonies (Borger 2005) and spreads at a constant rate (4.0 cm²/month) (Cervino et al. 2001).

Currently, little is known about the cause of DSD and its mechanisms of dispersal. One study found a bacteria strain in samples of DSD-infected corals but could not successfully inoculate healthy corals with this bacteria (Gil-Agudelo et al. 2004). Another study postulated that DSD could be a stress response rather than a pathogenic disease (Borger 2005).

Assuming that DSD is a pathogenic disease, investigating the existing hypotheses about coral disease transmission via water currents or biological vectors should provide insight into the mechanisms of DSD infection. These different potential dispersal mechanisms would be expected to cause different spatial distributions of DSD. Therefore, we sought to test these hypotheses by examining the spatial distribution of DSD-infected and healthy Massive Starlet Coral colonies on up-current and down-current sites on Little Cayman Island. We investigated infection through presence or absence of disease, percent of tissue infected

of each colony, and the area of discolored tissue of each colony. Since Cervino et al. (2001), demonstrated that DSD spreads at a constant rate, we assumed that area of discolored tissue is a proxy for time since infection. If DSD is transmitted through the water column via currents, it should occur more frequently or in a more advanced state on the up-current side of the island. If biological vectors are an important transmission mechanism, DSD should occur in clusters corresponding to the smaller-scale range of the vector organisms. A combination of large-scale and small-scale spatial patterns would point to both transmission mechanisms playing a role.

If our spatial analysis determines a random distribution of DSD, this would suggest that DSD is not a pathogenic disease but rather a response to stresses, such as warming and acidification. Though we lacked the means to measure these global stressors directly, we did measure hyperplasm and encroaching algae as potential indicators of stressful conditions. Hyperplasm forms as a response to stressors such as high ultraviolet radiation or algal or fungal agents (Humann and Deloach 2002). Encroaching algae competes with coral for space and can release chemicals that can kill corals (Rasher et al. 2011). A relationship between disease and these stress indicators, as well as a random distribution of DSD on both large and small spatial scales would support the hypothesis that DSD is a symptom of stress. However, no relationship between disease and hyperplasm and encroaching algae does not exclude the possibility that DSD is a stress response rather than a pathogenic disease.

METHODS

Study Site

The study sites were located on Little Cayman Island, which is located in the central Caribbean, separated from other landmasses



Fig 1. Massive Starlet Coral (*S. siderea*) displaying symptoms of Dark Spot Disease (from <http://www.masna.org/portals/0/NOAACoralDisease/dsd.htm>).

by deep ocean channels. It has very little development and no agriculture, and both study sites were located in Marine Parks that were established in the mid-1980s (Coelho and Manfrino 2007). Fishing pressure and other anthropogenic stressors are relatively low, and the reefs are among the most pristine in the Caribbean. The prevailing current comes from the northeast in the winter and from the southeast in the summer (Rob Hedges, *pers. comm.*). Therefore, the prevailing current runs east-west throughout the year.

We sampled at two sites on Little Cayman Island, Grape Tree Bay and Preston's Bay. Grape Tree Bay is on the north side of the island and further east than Preston's Bay, on the southwest side of the island. Thus, prevailing currents would likely hit Grape Tree Bay before Preston's Bay. The reefs in

both sites are comprised of a fringing reef with small patch reefs in the back reef.

Massive Starlet Coral and Boulder Star Coral both exhibit DSD in these sites. The discoloration associated with DSD appears both around the edges of coral colonies and in spots in the middle of the colony (Fig. 1). Both species exhibit hyperplasm, a growth abnormality in which polyps are enlarged. For this study, we only surveyed Massive Starlet Coral.

Data Collection

On February 26-27, 2012, we measured the abundance and distribution of Massive Starlet Coral colonies in Grape Tree Bay and Preston's Bay on Little Cayman Island. At each site, we haphazardly chose a starting location and swam in one direction, stopping at every Massive Starlet Coral colony we encountered. At Grape Tree Bay, the starting location was the western edge of the Little Cayman Research Center. At Preston's Bay, the starting location was the dock. At each site, we sampled a linear distance of approximately 275 meters. At each Massive Starlet colony, we recorded the distance and cardinal direction from the previous colony such that the colonies could be mapped. For each colony, we also recorded the length and width of the aerial view of the colony, the presence or absence of hyperplasm, a visual estimate of the percent coverage of DSD, and a visual estimate of the composition of the environment at each colony's borders (the percent of sand, algae, coral, encrusting sponge, and seagrass along the colony's circumference). At each site, we recorded water temperature over a 24-hour period using a HOBO TidbiT v2 temperature logger (Onset, Bourne, MA).

Statistical Analyses

We performed a t-test to compare the mean percent of disease of each colony between the two sites and a contingency analysis between site and presence or absence of DSD. To compare the time since infection between sites, we calculated the diseased area of each colony by multiplying percent disease by the area of the colony. Because DSD progresses at a constant rate regardless of colony area (Gil-Agudelo et. al 2004), the diseased area of the colony can be used as an indication of the time since the colony was infected. We performed a t-test comparing the average natural log of diseased area of each colony between the two sites, excluding colonies with 100 percent disease coverage (for which infected area no longer increases with time) and unaffected colonies. We performed a contingency analysis between presence of hyperplasm and presence of disease. We performed a correlation matrix between percent coverage of DSD affecting the colony, percent of the colony's perimeter as algae, and the area (length x width) of the colony. These analyses were performed using JMP 9.0 software (SAS Institute, Cary, NC).

Spatial Analysis

We converted the spatial relationships between colonies into a cartesian coordinate system using the equations $x = r \cos \theta$ and $y = r \sin \theta$, where r is the distance between two colonies and θ is the compass heading converted to radians. We used Quantum GIS 1.7.0 software to map the colonies of Massive Starlet Coral at each site (Open Source Geospatial Foundation, Beaverton, OR). We used Moran's I to test for spatial clustering of percent disease of colonies and size of disease patches (Moran 1950).

RESULTS

From 10:00 on 27 February to 10:00 on 28 February, average water temperature was 27.1°C at Grape Tree Bay and 27.4°C at Preston's Bay.

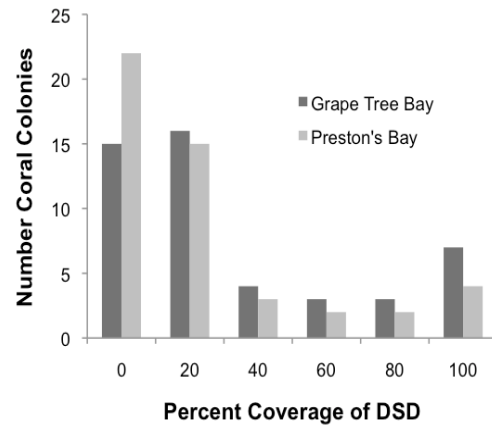


Fig. 2. Percent cover of Dark Spot Disease on Massive Starlet Coral colonies at Grape Tree Bay and Preston's Bay.

On 27 and 28 February, we collected data for 48 colonies of Massive Starlet Coral on each side of Little Cayman ($N_{\text{total}} = 96$). Massive Starlet Coral colony areas ranged from 130 cm² to 138,250 cm² and the mean colony area was not significantly different between sites ($t_{93.85} = -1.82$, $p = 0.0723$). 61.5% of all Massive Starlet Coral colonies at both sites exhibited symptoms of Dark Spot Disease (Fig. 1). However, most affected corals had less than 30% disease cover (Fig. 2). There was no significant difference in number of afflicted colonies between the two sites (Pearson's $\chi^2 = 2.15$, $p = 0.14$, $N = 96$; Fig. 2). There was also no significant difference between percent of disease coverage on each colony between the two sites ($t_96 = -1.38$, $p = 0.17$). However, Grape Tree Bay exhibited larger areas of afflicted tissue on each colony than Preston's Bay ($t_96 = -2.26$, $p = 0.027$, Fig. 3, Fig. 4).

We found that there was no clustering of percent cover of Dark Spot Disease among coral colonies at either site (Grape Tree Bay: $I = -0.04$, $z = -0.85$, $n = 48$, $p = 0.40$; Preston's Bay: $I = -0.03$, $z = 0.78$, $n = 48$, $p = 0.44$; Fig. 5).

Table 1. Correlation matrix between percent DSD cover, percent algae surrounding each colony, and the area (log) of each Massive Starlet Coral colony. None of the factors were correlated.

	Percent Disease Cover	Percent Algae Cover	Area (log)
Percent Disease Cover	1	-	-
Percent Algae Cover	-0.0041	1	-
Area (log)	0.0312	-0.0501	1

Additionally, we found no clustering of area of diseased coral among colonies at either site (Grape Tree Bay: $I=-0.02$, $z=0.16$, $n=48$, $p=0.87$; Preston's Bay: $I=-0.02$, $z=-0.13$, $n=48$, $p=0.90$; Fig. 4).

We found that presence of hyperplasm did not relate to the presence of disease (Pearson's $r^2=0.778$ $P=0.37$, $n=96$) or percent cover of disease ($t_{96}=1.13$, $p=0.28$). We found no significant relationships in the correlation analysis between percent algae cover, percent disease cover, and area (Table 1).

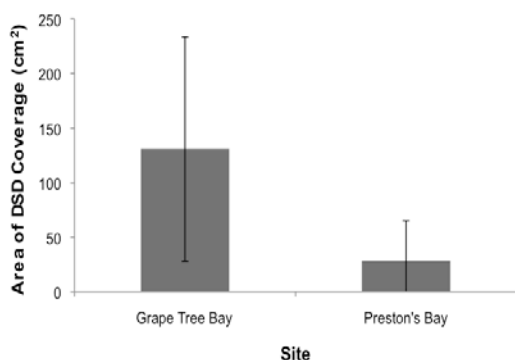


Fig 3. Massive Starlet Coral colonies at Grape Tree Bay exhibited greater areas of Dark Spot Disease infected tissue per colony than colonies at Preston's Bay, when excluding colonies completely infected ($t_{54}=-2.26$, $p=0.027$). Bars show 1 standard error.

DISCUSSION

DSD affects Massive Starlet Coral colonies on both sides of the island. However, because DSD spreads across tissue at a uniform rate (Cervino et al. 2001), the greater area of

diseased coral tissue in Grape Tree Bay suggests that DSD infected colonies in Grape Tree Bay before those in Preston's Bay. Given that Grape Tree Bay is hit by the prevailing current before Preston's Bay, this result supports the water current transmission hypothesis.

The non-clustered distribution of DSD within sites does not support the biological vector transmission hypothesis. However, one possible explanation for the random distribution of DSD within sites could be that all Massive Starlet Coral colonies have been exposed to DSD, but some are susceptible to infection while others are not, as evidenced by the coral colonies that had no DSD. Corals that are susceptible may be more likely to show symptoms of the disease, while corals that are not susceptible may be more likely to remain healthy. Differences in genetics or environment could render some colonies more disease-prone (Gochfeld et al. 2006).

Alternatively, the random distribution of DSD within sites could support the hypothesis that DSD is a stress response rather than a pathogenic disease and that local stressors cause the symptoms of DSD. Though the stress indicators we measured (hyperplasm and encroaching algae) did not correlate with disease presence or percent cover of DSD, we cannot rule out the possibility that the symptoms of DSD are a response to other local stressors that we did not measure. Additionally, global stressors such as warming and acidification could be affecting

coral around Little Cayman. These global stressors may play a proportionally larger role, given that Little Cayman Island is a pristine environment where anthropogenic stressors such as runoff, sedimentation, and overfishing are minimized. We suggest that further research should investigate other potential stressors and whether they result in

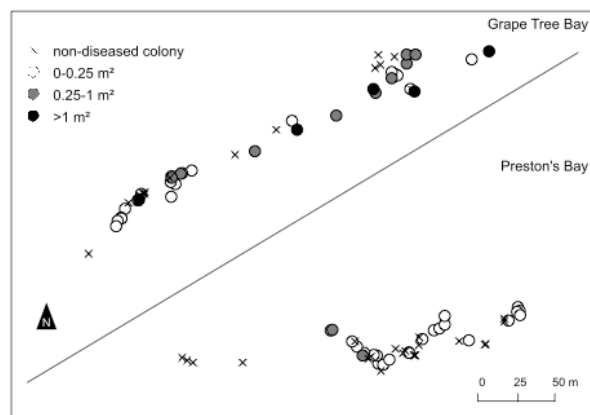


Fig 4. Map of distribution of area of diseased Massive Starlet Coral tissue at Grape Tree and Preston's Bay, Little Cayman Island. Each circle represents one Massive Starlet Coral colony.

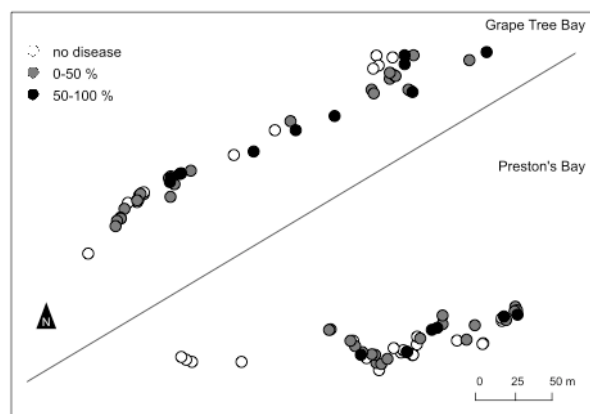


Fig 5. Map of distribution of percent disease coverage on Massive Starlet Coral colonies at Grape Tree and Preston's Bay, Little Cayman Island. Each circle represents one Massive Starlet Coral colony.

DSD symptoms.

Regardless of whether DSD is a pathogenic disease or a response to stress, our results corroborate the perceived increase of

DSD throughout the Caribbean. In 1999, Coelho and Manfrino (2009) documented no cases of DSD on reefs surrounding Little Cayman and by 2004, still only recorded symptoms on 3.9% of all coral colonies. Our study measured DSD on a staggering 63% of Massive Starlet Coral colonies. Furthermore, the current DSD coverage on Massive Starlet Coral in Little Cayman exceeds the DSD coverage of Massive Starlet Coral in 1998 at any of the 5 Caribbean sites investigated by Cervino et al. (2001), despite the relatively pristine conditions of Little Cayman reefs. The increasing prevalence of DSD in Caribbean coral reefs raises questions about how anthropogenic impacts may be exacerbating coral disease and degradation. Since so little is understood about DSD and other coral diseases, it is all the more important to make conscientious efforts to reduce anthropogenic impacts on coral reefs because we remain dangerously unaware of the consequences.

ACKNOWLEDGEMENTS

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FACTORS AFFECTING FISH DIVERSITY ON CORAL REEF “ISLANDS”

M. Hemayat R. Chowdhury, Shea E. Flanagan, and Nina B. Frankel

Faculty Editor: Celia Y. Chen

Abstract: Recent changes in coral reef ecosystems caused by natural and anthropogenic factors affect fish diversity. By determining the causes of changes in fish diversity, predictions can be made about how best to minimize species loss in the face of coral reef damage. We examined coral patches in a Caribbean back reef to determine if fish diversity on small-scale coral “islands” conformed to the predictions of the island biogeography theory. We supplemented the theory’s predictions of diversity by including percent coral cover as a metric of island quality. We examined the effects of island volume, island distance from the main reef, and percent coral cover on total and territorial reef fish abundance and species richness. We found that as island volume increased, total fish abundance and species richness increased, and territorial species richness trended to increase. Percent coral cover and distance from main reef did not significantly predict abundance or species richness neither for territorial nor total species. When coral cover decreased, the proportion of algal turf farmers to total fish abundance increased, suggesting that these species may somewhat compensate for the loss of species that obligately depend on coral. The importance of island volume in predicting abundance and species richness suggests that the size of patch reef islands is a factor that should be considered when marine reserves are established.

Key Words: coral decline, island biogeography theory, coral reef fish diversity

INTRODUCTION

Coral reef ecosystems are highly sensitive to change but have persisted across geologic time. Recently, however, they undergone significant abiotic and biotic changes due to both natural and human-induced causes (Anthony et al. 2008, Hoegh-Guldberg et al. 2007). These changes could consequently affect fish diversity. The loss of fish diversity on coral reef ecosystems has implications beyond homogenization of the reef fish community (Mumby and Steneck 2008). Such a decline would likely affect ecosystem services such as fisheries and tropical tourism and in turn local economies (Barbier et al. 2011). Through determining what factors influence fish biodiversity in coral reefs, predictions can be made about how best to minimize fish species loss in the face of reef damage.

Island biogeography theory assumes that organisms inhabit hospitable patches

separated by inhospitable spaces and predicts that organism diversity on islands is based on size and distance to the mainland. Larger islands and islands closer to the mainland are predicted to have greater diversity due to greater availability of niches and likelihood of gene flow from the mainland, respectively (MacArthur and Wilson 1967). These patterns have been demonstrated to predict reef fish diversity at the scale of entire Caribbean island nations (Sandin et al. 2008). Caribbean reef fish diversity has conformed to the predictions of the island biogeography theory in previous studies (Sandin et al. 2008).

Islands can also be examined on a smaller scale. If large contiguous stretches of coral reefs are considered as analogous to the ‘mainland,’ smaller reef patches may be the equivalents of ‘islands.’ In addition to the size of an island and its distance from the mainland, island quality could play an important role in predicting fish biodiversity. One metric of island quality is the percent

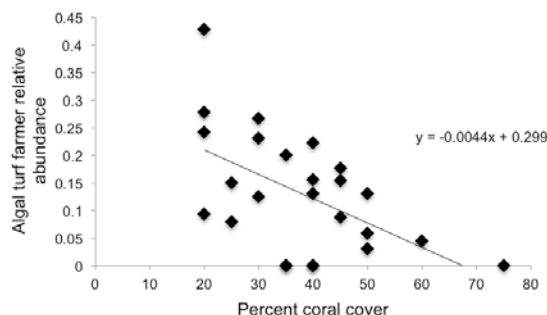


Fig. 1. The relative abundance of algal turf farmers (number of turf farmers/number of total fish) as a function of percent coral cover on each coral island.

coral cover of an island. We sought to determine how island size, distance from the main reef, and quality affect coral reef fish abundance and species richness. Since island biogeography theory assumes that populations are at equilibrium, we focused on the abundance and species richness of territorial reef fish, as they are permanent coral island residents. We hypothesized that both total and territorial fish abundance and species richness would increase as island volume increased, percent coral cover increased, and distance from the main reef decreased.

Table 1. Factors influencing territorial fish abundance of coral

Effect	DF	Sum of Squares	F	P
Distance (m)	1	1.08	0.05	0.818
Island volume (m ³)	1	55.20	2.79	0.112
Percent coral cover	1	13.68	0.69	0.417
Distance (m) × Island volume (m ³)	1	46.91	2.37	0.141
Distance (m) × Percent coral cover	1	13.44	0.68	0.421
Island volume (m ³) × Percent coral cover	1	6.65	0.34	0.569

METHODS

On 26 and 27 February 2012, we surveyed coral “islands” ($n = 25$) in the back reef at Jackson Point (a marine protected area) on

Little Cayman Island. An island was defined as a patch of coral reef separate from the main fringing reef. Each island sampled was at least two meters from the nearest coral growth. At each island, we performed a visual census to determine the abundance of each fish species present. We remained at each island for approximately 20 minutes around midday. For fish species with distinct juvenile and adult phases, we recorded the abundance of juveniles and adults separately. We then measured each island’s height from the sea floor, width, length, and distance from the main reef using transect tape. We used each island’s height, width, and length to calculate the volume of the island by treating each island as an elliptical cylinder with length and width as the semimajor and semiminor axes, respectively. At each island, we visually estimated percent coral cover.

We used multiple regressions to analyze the effects of island volume, island distance from main reef, and percent coral cover on both total and territorial fish abundance and species richness at each island. We used species richness as a metric of fish diversity. Additionally, we examined the relationships between percent coral cover and abundance of corallivores, grazers and algal turf farmers. We used linear regressions to analyze the relationships between percent coral cover and abundance of these functional feeding groups. All analyses were performed using JMP 9.0 (SAS Institute, Cary, NC).

RESULTS

All surfaces on coral islands that were not covered by coral were covered by algae. The proportion of algal turf farmers over total fish abundance decreased with increased percent coral cover ($r^2_{24} = 0.30$, $F = 9.87$, $P = 0.005$; Fig. 1).

Table 2. Factors influencing territorial species richness on coral islands.

Effect	DF	Sum of Squares	F	P
Distance (m)	1	0.57	0.32	0.582
Island volume (m ³)	1	7.33	4.08	0.058
Percent coral cover	1	0.05	0.03	0.872
Distance (m) × Island volume (m ³)	1	2.49	1.39	0.254
Distance (m) × Percent coral cover	1	0.49	0.27	0.609
Island volume (m ³) × Percent coral cover	1	0.14	0.08	0.780

No measured variables significantly correlated with territorial fish abundance or territorial species richness, but territorial species richness tended to increase with island volume (Table 1; Table 2, respectively). Total fish abundance and species richness increased with island volume (Table 3; Table 4, respectively). Total fish abundance tended to increase with island distance from the main reef, although not significantly (Table 3).

DISCUSSION

Our results suggest that fish species richness loosely conforms to the predictions of island biogeography theory. The trend of greater territorial species richness with island volume followed the predictions of island biogeography theory. When all species were considered, species richness significantly increased with island volume. Larger coral islands not only offer more habitat and food availability to resident species, but may also offer a higher frequency of encountering prey

Table 3. Factors influencing total fish abundance on coral islands.

Effect	DF	Sum of Squares	F	P
Distance (m)	1	359.00	4.23	0.055
Island volume (m ³)	1	1146.59	13.51	0.002
Percent coral cover	1	0.48	0.01	0.941
Distance (m) × Island volume (m ³)	1	264.60	3.12	0.094
Distance (m) × Percent coral cover	1	37.24	0.44	0.516
Island volume (m ³) × Percent coral cover	1	7.51	0.09	0.770

Table 4. Factors influencing total species richness on coral islands.

Effect	DF	Sum of Squares	F	P
Distance (m)	1	0.02	<0.01	0.965
Island volume (m ³)	1	55.33	6.01	0.025
Percent coral cover	1	0.12	0.01	0.911
Distance (m) × Island volume (m ³)	1	16.18	1.76	0.201
Distance (m) × Percent coral cover	1	1.88	0.20	0.657
Island volume (m ³) × Percent coral cover	1	6.38	0.69	0.416

for species that visit the island, increasing overall species richness. Since distance from the main reef did not predict species richness for either territorial species or total species, it seems that coral islands do not have the same degree of isolation that restricts both movement and gene flow as the islands described in MacArthur and Wilson's (1967) island biogeography theory.

The factors that we found to drive fish abundance differ from those that drive species richness. Interestingly, territorial fish abundance did not increase with island volume but total fish abundance did, which suggests that competition among territorial fish may decrease the number of territorial fish that could utilize the increased habitat and food sources potentially provided by larger islands. However, fish that visit the island but do not establish territories are still able to take advantage of food sources provided by the island, which could explain why more fish overall were found at bigger islands. The trend of total fish abundance increasing with distance from main reef could potentially be explained by a higher predator presence near the main reef (Lobben and Cheek 2008) and larger predators being less likely to venture closer to shore. Additionally, since the main reef is nutrient-poor, fish may be attracted to islands that are closer to the nutrient-rich sea grass beds.

Contrary to our expectations, percent coral cover did not predict fish abundance

nor species richness. One possible explanation for this finding is that algal patches provide food or habitat sources for many fish, which could partially compensate for the structure and safety that coral would have provided. This idea is supported by our finding that the proportion of algal turf farmers to total fish increased with decreased coral cover, and thus may have increased algal cover. Future studies should investigate if fish abundance and species richness are more dependent on the presence of specific coral species, rather than percent coral coverage.

Furthermore, low percent coral cover could indicate general coral decline in this reef. Corals are declining globally at an alarming rate, which is a major cause for concern because their loss will likely be detrimental to fish diversity and overall marine biodiversity (Munday et al. 2008). Causes of coral decline are both chronic and acute, including bleaching, ocean acidification, warming, storms and disease (Mumby and Steneck 2008). In many areas, coral is being supplanted by macroalgal growth (Rasher et al. 2011). Our study sheds light on the factors influencing fish diversity, which could help determine how best to mitigate fish species loss due to coral loss.

The importance of volume in predicting abundance and species richness suggests that the size of patch reef islands is a factor that should be considered when establishing marine reserves. Studies that examine factors affecting fish diversity, are important components in forming optimal management strategies for the preservation of biodiversity in the face of global coral decline.

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REPLENISHMENT ZONES ARE EFFECTIVE FOR CONSERVATION OF *STROMBUS GIGAS*

M. HEMAYAT R. CHOWDHURY, ANNA L. DEFFEBACH, AND JESSE T. RIEB

FACULTY EDITOR: CELIA Y. CHEN

Abstract: Marine protected areas are an important tool for protecting marine species from overfishing. One potential function of these areas is to protect habitat for source populations that replenish populations in areas where fishing is allowed. In this study, we investigated the effects of one such protected area, South Hole Sound Replenishment Zone on Little Cayman Island, on the abundance and size distribution of Queen Conch, *Strombus gigas*. We sampled size, age structure, and density of *S. gigas* inside and outside the protected area. We estimated age using the Bertalanffy equation and also used life tables to model the future size of each population. We found that *S. gigas* in the protected area were younger and occurred at a higher density than those in the harvesting area. Our model showed that the population in the protected area will grow significantly faster than that in the harvesting area over the next ten years. Our density measurements suggest that the population in the harvesting area may be experiencing a decreased reproductive rate due to Allee effects, and that the population in the harvesting zone may be supplemented by spillover from the replenishment zone.

Key words: Allee effect, marine protected area, spillover, *Strombus gigas*, Queen Conch

INTRODUCTION

The success of marine protected areas in preserving endangered species has been widely debated. One common objective of Marine Protected Areas (MPAs) is to protect populations of commercially exploited species so that their numbers do not fall below those needed to sustain a population (Béné and Tewfik 2003). It is assumed that individuals may then migrate into fished areas from the protected areas (spillover effect), or eggs and larvae from protected areas may disperse into fishing areas (recruitment effect) and thereby support sustainable populations. It is essential to determine the effectiveness of MPAs in maintaining healthy populations of commercially exploited species.

Overfishing has decimated commercially important fish populations throughout the Caribbean, and many countries have established MPAs in an effort to conserve these populations (Roberts and Polunin 1993).

Multiple small MPAs were created in the Cayman Islands in 1987 to protect populations of over harvested marine organisms (CIDE 2011). A replenishment zone for the highly exploited Queen Conch (*Strombus gigas*) was created in South Hole Sound 25 years ago (CIDE 2011). Due to declining populations, in 2007 a stricter harvesting policy was enacted to preserve populations of *S. gigas* (CIDE 2010). *S. gigas* are protected within the replenishment zone year round, but are subject to harvesting in other unprotected areas of South Hole Sound during the non-breeding season (November through May) (CIDE 2010). *S. gigas* harvesting limits are set at ten individuals per boat per day or five *S. gigas* per person per day, whichever is less (CIDE 2010). However, harvesting regulations are not strictly enforced (Lowell Forbes, pers comm.)

Evaluation of the population dynamics of *S. gigas* is of significance as they are economically important organisms that have experienced large populations declines

throughout the Caribbean (Stoner 1996). Conservation of *S. gigas* is complicated by the fact that their populations are subject to Allee effects, where, at low densities, a negative relationship between density and reproductive rate creates positive feedbacks that may lead to extinction of the population (Stoner et al. 2012). Additionally, *S. gigas* require four years to reach maturity resulting in slow population recovery times (Stoner et al. 1996).

We sought to determine whether the South Hole Sound Replenishment Zone was successful in maintaining a population of *S. gigas*. We surveyed and compared their abundance and size distributions within the replenishment zone and the adjacent harvesting area. We hypothesized that we would find (1) greater population density in the replenishment zone, (2) a difference in age structure between the replenishment and unprotected zones (i.e. more young juveniles in the replenishment zone), (3) the presence of more individuals in younger age classes in the replenishment zone leading to a larger future reproductive population and therefore a higher population growth rate, and (4) smaller adult *S. gigas* in the fishing zone, as larger individuals would experience greater harvesting pressure.

METHODS

We surveyed populations of *S. gigas* on 2, 3 and 4th March, 2012 at two sites in South Hole Sound, Little Cayman. One site was within the replenishment zone and the other was in the adjacent harvesting zone (Fig. 1). While both zones contained a variety of substrates, both sites in this study were of similar substrate composition (sandy with algal patches), depth (1-1.5 m) and distance from the fringing reef (100-175 m).

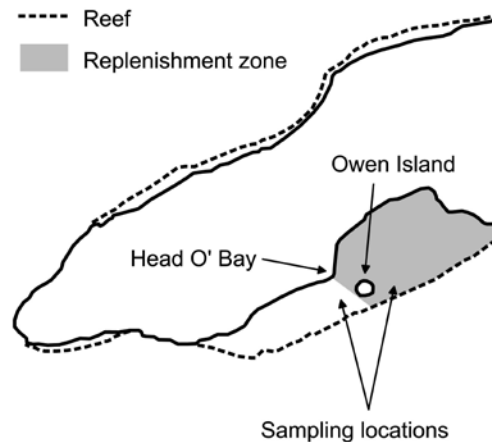


Fig 1. Map showing approximate sampling locations in South Hole Sound, Little Cayman Island. The shaded replenishment zone is protected from harvesting.

At each site we surveyed 10 40x2 m transects that were approximately parallel to one another, but separated by about 10 m. We determined the age class of each individual by noting the shell morphology (adult shells have a flared outer lip, Appeldoorn 1998), and recorded the shell length by measuring the length from the siphonal groove to the apical spiral (Fig. 2). Additionally, we noted whether an individual was alive or dead and if it showed evidence of human consumption as indicated by hammer damage between the second and third spiral (Lowell Forbes, *pers comm.*).

Analysis

We used a Pearson's Chi-squared test to compare the distributions of dead and live, adult and juvenile, and age size classes of *S. gigas* between the two sites. We used two-way ANOVAs to compare density and length of adult and juvenile *S. gigas* between the two zones.

We used a Bertalanffy growth function:

$$\text{Age} = \frac{\ln\left(1 - \frac{l}{0.95(l_{\max})}\right)}{-k} + t_0,$$

where l is the length of the shell, l_{\max} is the length of the longest *S. gigas* measured, k is the growth coefficient, and t_0 is the age at $l = 0$ to estimate age of juvenile *S. gigas* from their length (Appeldoorn 1990). We obtained literature values of $k = 0.442$ and $t_0 = 0.09$ for a population of similarly-sized *S. gigas* (Appeldoorn 1990).

We used a life table to model projected *S. gigas* populations over ten years given the age structure that we observed. We used the exponential population growth equation $N_t = N_{t-1}e^r$ to estimate future population size (Appendix 1). We used estimated survivorship (l_x) for different ages using a model by Appeldoorn (1988). As we were unable to find information on mortality rates of *S. gigas* larvae, we manipulated different reproductive rates (m_x) for adult *S. gigas* and analyzed the effect on the population for ten years (at values of 10, 30, and 3000 individuals · individuals⁻¹ · year⁻¹).

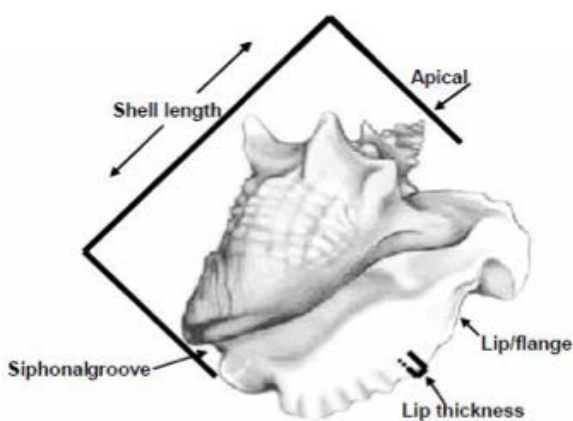


Fig 2. Diagram showing measurement locations for mature *Strombus gigas* (Collins and Harrison 2007).

RESULTS

S. gigas were found at a significantly higher density in the protected area than in the harvesting area ($F_{3,33} = -5.14$, $p < 0.001$; Fig. 3). There was no main effect of age class on density nor an interaction between area and age class ($F_{3,33} = 1.55$, $p = 0.13$; $F_{3,33} = -1.12$, $p = 0.23$, respectively). Mean density of adults was 238 *S. gigas* · ha⁻¹ in the harvesting area and 1013 *S. gigas* · ha⁻¹ in the protected area. Mean density of all live *S. gigas* was 388 *S. gigas* · ha⁻¹ in the harvesting area and 1738 *S. gigas* · ha⁻¹ in the protected area.

83 percent of sampled shells contained live *S. gigas*, and the proportion of live and dead *S. gigas* was not different between the two areas (Pearson's $\chi^2 = 1.35$, $p = 0.25$, $n = 202$). Of the 202 *S. gigas* sampled, only five showed signs of human consumption.

There was not a significant difference in the relative proportions of juveniles and adults in the two areas (Pearson's $\chi^2 = 0.114$, $p = 0.74$, $n = 165$). Mean *S. gigas* length was higher in the harvesting area ($F_{3,161} = 8.07$, $p = 0.005$; Fig. 4). There was no significant interaction between age class and area ($F_{3,161} = 1.93$, $p = 0.17$). Adult mean shell length was 20.9 cm in the replenishment zone and 21.6 cm in the harvesting zone. The mean shell length for juveniles in the replenishment zone was 16.0 cm and 17.8 cm in the harvesting zone. The smallest juvenile observed in the replenishment zone was 9.5 cm, while the smallest juvenile observed in the harvesting zone was 13 cm (Fig. 5). When juvenile length was used to estimate age, the mean age of juveniles was 2.31 years in the protected area and 2.88 years in the harvesting area.

Modeling *S. gigas* life tables showed that with reproductive rates ranging from 10 to 3000 individuals · individual⁻¹ · year⁻¹, the *S. gigas* population in the harvesting area would be approximately four times smaller than that in the protected area after ten years.

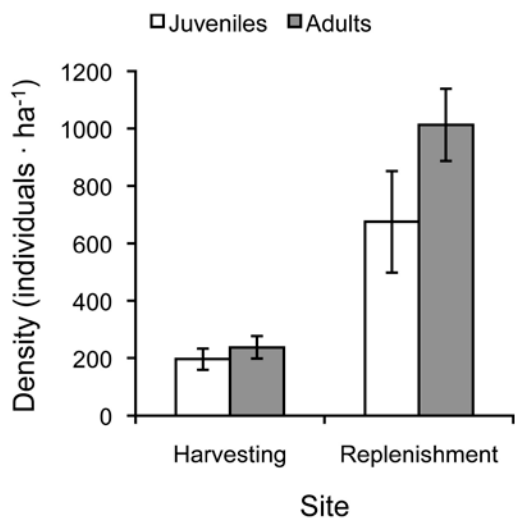


Fig 3. Mean density of adult and juvenile *Strombus gigas* in the harvesting zone and replenishment zone of South Hole Sound, Little Cayman. Error bars show one standard error.



Fig 4. Mean shell length of adult and juvenile *Strombus gigas* in the harvesting zone and replenishment zone of South Hole Sound, Little Cayman. Error bars show one standard error.

DISCUSSION

We found a significantly higher density of live adult *S. gigas* in the replenishment zone of South Hole Sound than in the unregulated (harvesting permitted) zone. A life table model for the *S. gigas* population in both sites suggests that with equal fecundity between the two sites, the population in the harvesting zone will remain at least four times smaller than that of the replenishment zone. This density difference may be exacerbated by Allee effects in the harvested area. Stoner et al. determined that the ability of *S. gigas* to find mates and reproduce decreases at low densities (2012). A population at the density observed in the harvesting area may experience a 45-65 percent decrease in reproduction due to Allee effects, while the population in the replenishment zone has a high enough density that its reproduction is unlikely to be impacted.

Contrary to what we had expected, adults were not smaller in the harvesting

area, indicating that harvesting pressure on larger individuals is not selecting for smaller individuals. Juvenile *S. gigas* were larger in the harvesting area than in the protected area. The size difference might be a result of fewer young *S. gigas* in the harvesting area. This could be due to lower reproductive rates because of the Allee effect induced by overfishing of adults. Because *S. gigas* take approximately four years to reach sexual maturity, this could potentially have substantial negative impacts on future population growth. The size difference could indicate that there is very little or no reproduction occurring in the harvesting area and that this population is maintained through spill over of more mature individuals from the replenishment zone. This explanation is supported by the fact that we saw no juveniles less than 13 cm long in the harvesting zone.

This difference in size could be the result of density dependence. Béné and

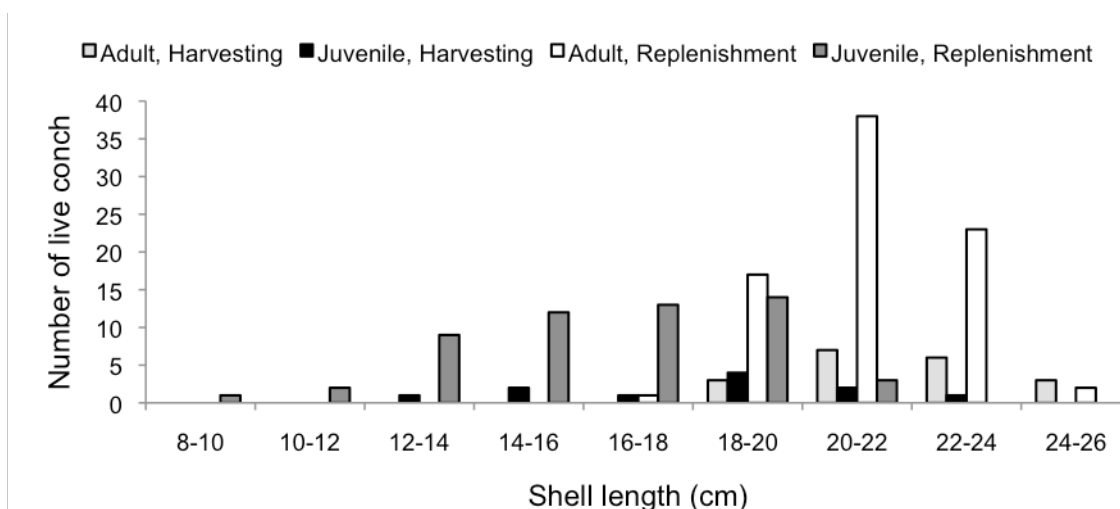


Fig 5. Frequency distribution of shell size of adult and juvenile conch in a protected replenishment zone and a harvesting area.

Tewfik (2003) observed decreases in individual growth rate at densities of 3778 individuals \cdot ha⁻¹. While we cannot exclude the possibility that density dependence was limiting growth, the highest densities observed in our study were considerably lower.

We found no difference in proportion of dead *S. gigas* between the two sites, suggesting that natural mortality may not affect one site disproportionately. The very low frequency of finding shells that had been harvested by humans and thrown back suggests that these shells are unlikely to have any significant influence on visual population estimates.

This study was conducted four months into the six-month harvesting season, and the ongoing harvesting may have affected the distribution of adult *S. gigas*. During the part of the year when harvesting is not allowed, spillover of conch from the replenishment zone into the harvesting zone may create a more even distribution. Future studies should examine whether *S. gigas* populations fluctuate with seasonality.

Given that the densities of both populations sampled are among the highest observed in the Caribbean (Stoner et al. 1996; Béné and Tewfik 2003) the *S. gigas* population at Little Cayman Island is remarkably strong. However, the differences in density between the two zones are still substantial. The great differences in density between such similar and nearby sites suggest that for organisms that do not move across large ranges a protected area can have a large impact on populations. We conclude that when attempting to conserve organisms with small range sizes, the creation of even a small protected area may be an extremely effective strategy.

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Appendix 1: Equations used for population growth projections.

$$(1) N_t = N_{t-1}e^{rt}$$

$$(2) r = \frac{\ln(R_0)}{G}$$

$$(3) R_0 = \sum_{x=0} l_x m_x$$

$$(4) G = \sum_{x=0} \frac{l_x m_x x}{R_0}$$

N = population size

r = intrinsic rate of increase (individuals · individual⁻¹ · year⁻¹)

t = time (years)

R_0 = net reproductive rate (individuals · individual⁻¹ · lifetime⁻¹)

G = generation time (years)

l_x = cumulative survivorship

m_x = fecundity at age x (individuals · individual⁻¹ that survive to the following year)

x = age class

INTERSTELLAR COMPETITION FOR SPACE: MECHANISMS FOR SPATIAL NICHE PARTITIONING IN TWO SPECIES OF *OPHIOCOMA*

WALES A. CARTER, MILO S. JOHNSON, BENJAMIN J. KESSLER, AND AMY E. VAN SCOYOC

Faculty editor: Celia Y. Chen

Abstract: The high diversity of coral reef ecosystems brings organisms of similar ecological tendencies into intimate association with one another, sparking competition and niche partitioning over limited food or spatial resources. *Ophiocoma* brittle stars, detritivorous suspension and deposit feeders, have been known to spatially partition their niches by occupying distinct microhabitat types. In this study, we used a range of light regimes as a proxy for differing microhabitats to investigate whether two species of brittle star (*Ophiocoma echinata* and *Ophiocoma wendtii*) differed in their microhabitat preference and whether displacement from their preferred microhabitat occurred as a result of interspecific interactions. Both species of brittle star were negatively phototactic, and there was no difference in microhabitat preference between species. However, when placed together at night, *O. wendtii* occupied darker areas more often than *O. echinata*. Furthermore, we found that *O. echinata* shared space with individuals of its own species significantly more often than it shared space with *O. wendtii*, whereas *O. wendtii* did not often share space with other individuals of either species.

Keywords: competition, luminosity regime, *Ophiocoma echinata*, *Ophiocoma wendtii*, niche separation

“As we lifted the rocks we were confronted by the snake-like, wild writhings of those fearsome devil-creatures, the brittle stars”

-Milo Johnson, 2012

INTRODUCTION

Coral reef ecosystems are hotspots of diversity in which species are forced to compete or specialize in order to acquire severely limited resources (Sale 1977). According to the competitive exclusion principle, organisms must differ in niche if they are to stably coexist (Hardin 1960). Although closely related species in regions of high diversity may appear to be identical in habitat, diet, and behavior, closer examination often reveals that these species differ subtly in their resource or microhabitat use. Spatial partitioning may be mediated by habitat preference or by competition in which a superior competitor forces the lesser species to occupy an inferior habitat (Sides and Woodley 1985).

Niche separation has been widely reported among several species of brittle stars, the most speciose family of echinoderms (Sides and Woodley 1985, Henkel and Pawlik 2005, Susman *et al.* 2009, Ruppert *et al.* 2004). It has been hypothesized that brittle stars, as night-foraging benthic detritivores, are more limited by space than food resources. (Kohn 1971). Brittle stars in the genus *Ophiocoma* have been known to spatially partition their habitats by occupying distinct crevice sizes (Sides and Woodley 1985). Since these organisms are also reported to be negatively phototactic (Hendler 1984), likely as an adaptation to escape predators, a difference in their degree of phototaxis may dictate which crevice sizes and microhabitat type they occupy.

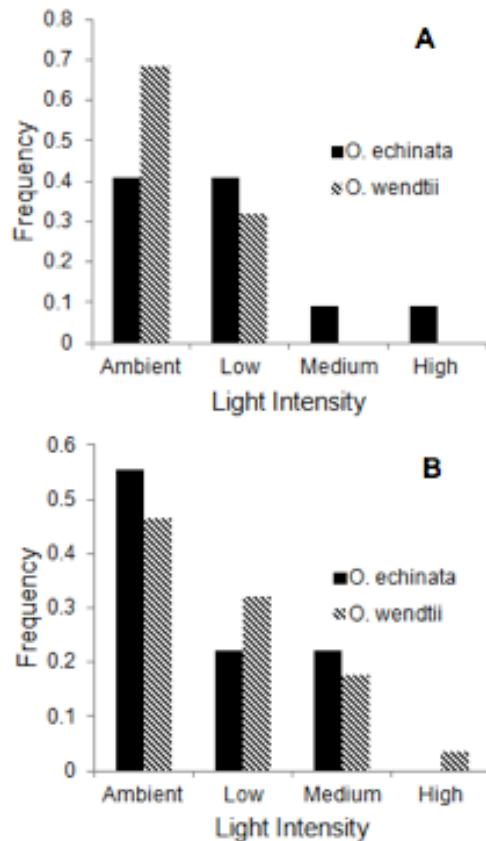


Fig 1. Frequency distribution of light-based site selection by *O. echinata* and *O. wendtii* each alone at night (A) and day (B). Light treatments ranged from ambient to high light intensity.

Ophiocoma wendtii and *Ophiocoma echinata* are abundant co-occurring brittle stars in the coral reef habitats of Little Cayman Island, which exhibit both negative phototaxis and spatial partitioning of microhabitat (Hendler 1984, Susman *et al.* 2009). Since it is unknown what mechanisms preserve spatial partitioning between these two species, we sought to investigate whether light intensity is a mechanism for differential microhabitat selection. We created microhabitats of artificial light and tested if *O. wendtii* and *O. echinata* differed in their microhabitat preference and whether they were displaced from this preferred

microhabitat in the presence of a competitor.

METHODS

On 2-3 March 2012, we collected brittle stars of the species *Ophiocoma echinata* (N=11) and *Ophiocoma wendtii* (N=11) from Grape Tree Bay on Little Cayman, BWI. We noted the species and recorded disc diameter at the widest point.

We constructed variable luminosity regimes by taping a Princeton Tec Torrent® LED flashlight to each corner of a white 12-quart washbasin filled with 3 cm of seawater. We left one flashlight at full intensity, covered one flashlight with 8 sheets of wax paper, one flashlight with 16 sheets of wax paper, and turned one flashlight off to create a light gradient from bright to dark respectively.

To measure brittle star preference for sites of differing light intensities, we placed a single brittle star in the center of the tank and waited until it remained in one position for more than 30 seconds after an initial 2 minutes. We then noted the light level it occupied. We repeated this experiment 3 times at day and 3 times at night for each individual of *O. echinata* and *O. wendtii*.

We also measured each individual brittle star's light intensity preference in the presence of a single individual of its own species or the other species. We placed both individuals in the tank simultaneously and recorded the light level each moved to and remained in for more than 30 seconds after an initial 2 minutes for each trial, and noted whether the two individuals were touching. We

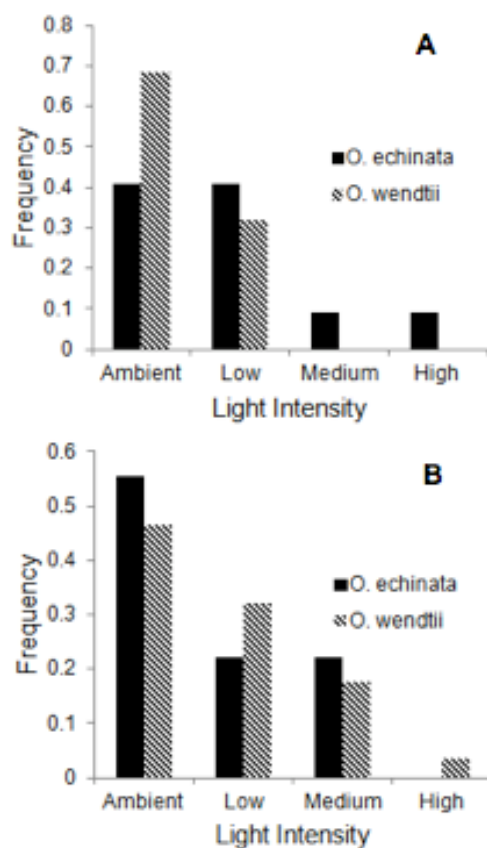


Figure 2. Frequency distributions of light-based site selection by *O. echinata* and *O. wendtii* when paired with a member of the opposite species at night (A) and during the day (B). Light treatments ranged from ambient to high light intensity.

periodically rearranged the lights of the washbasin.

Data analysis

We used an ordinal logistic regression to test the effects of species and time on preferred light intensity. We also used a one-way ANOVA to investigate the effect of an individual's size on light level preference. To test for differences in preference of light intensity when a brittle star was in the presence of another individual, either of the same species or the other species, we used contingency analyses for both night and day. We also

used contingency analyses of a binary variable of whether or not the two individuals were touching and the species to examine whether species exhibited interspecific or intraspecific separation in the tank. Finally, we used contingency analyses for all interspecific and intraspecific trials to investigate whether the size of the individual influenced an individual's light level preference.

RESULTS

We found that *Ophiocoma echinata* and *Ophiocoma wendtii* did not differ in their light level preference either during the day or at night ($\chi^2=5.17$, $df=3$, $P=0.16$). In the absence of other individuals, both species preferred sites with a lower light intensity (Figure 1a and b). We found that size of the individual was not related to light level preference for either species (*O. echinata* $F_{3,39} = 0.96$, $P=0.42$; *O. wendtii* $F_{3,49} = 0.05$, $P=0.98$).

During tests of light level selection in the presence of the other species that were conducted at night, *O. wendtii* selected for low light intensities, whereas *O. echinata* occupied sites with higher light intensity ($\chi^2=13.63$, $df=3$, $P=0.003$; Figure 2). However, there was no significant difference when the same trial was performed during the day ($\chi^2=1.19$, $df=3$, $P=0.76$; Figure 2). By contrast, when we tested light level selection in the presence of the same species, we found that *O. echinata* selected sites with low light intensity, while *O. wendtii* was more uniformly distributed between light intensities ($\chi^2 = 17.8$, $df=3$, $P < 0.001$; Figure 3). We also found that *O. echinata* shared space (were in physical contact at the end of the trial) with individuals of its own

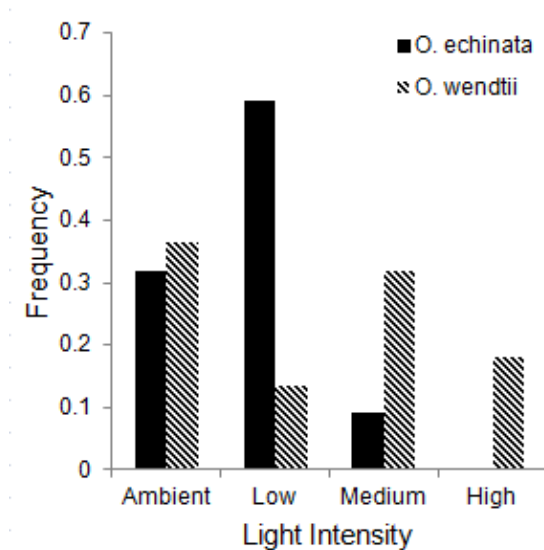


Figure 3. Frequency distribution of light-based site selection by *O. echinata* and *O. wendtii* when paired with a member of the same species. Light treatments ranged from ambient to high light intensity. *Ophiocoma echinata* clumped at ambient light sites, while *O. wendtii* was distributed more uniformly.

species significantly more often than it shared space with *O. wendtii* when placed in the tank together ($\chi^2 = 6.72$, $df=1$, $P=0.01$). However, we found that *O. wendtii* did not share space with any conspecifics more often than the other species ($\chi^2=0.25$, $df=1$, $P=0.67$). Finally, we found no significant relationship between brittle star size and light level preference for any interspecific or intraspecific trial.

DISCUSSION

In accordance with previous studies on other ophiuroids, *Ophiocoma echinata* and *Ophiocoma wendtii* preferred darker areas, a behavior which most likely represents an adaptation for selecting microhabitats that offer protection from predators (Hendler 1984, Drolet *et al.* 2004). The two species' light preferences when alone were strikingly similar, so differential light preference is most likely not a mechanism for the reported

microhabitat niche separation (Sides and Woodley 1985, Susman *et al.* 2009). It is possible that another mechanism for identifying suitable habitats, e.g. a tactile preference, differs between the two species, but since light sensitivity has been reported as a highly important sense associated with brittle star behavior, similar light preferences may indicate similar microhabitat preferences (Hendler 1984).

If the two species do prefer the same microhabitats, interspecific competition could provide a potential mechanism for the niche separation observed in sympatric populations (Sides and Woodley 1985, Susman *et al.* 2009). Though removal or addition experiments would be necessary to show whether competition occurs between these two species in nature, we see some evidence for displacement of *O. echinata* by *O. wendtii* in our experiment. First, *O. echinata* was much less likely to end a trial in physical contact with *O. wendtii* than with another *O. echinata*. *O. wendtii* also displaced *O. echinata* from the preferred areas when together during the night. The fact that we did not observe the same result during the day could potentially be explained by diel activity patterns; *Ophiocoma* are simply more active at night, and perhaps are more likely to displace other individuals from preferred areas during their active phase (Hendler 1984).

Though these two *Ophiocoma* species are similar in morphology, diet, and light-level preferences when tested alone, our observations of the two species interacting revealed differences in behavior. Even if *O. wendtii* does displace *O. echinata* from preferred microhabitats in nature as it did in our trials, the niches of these species

likely differ in yet undiscovered ways, allowing them to persist in each other's presence. These kinds of subtle differences may allow countless species to flourish and survive in the furiously competitive and wildly diverse world of coral reefs.

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ALLOMETRIC SCALING OF MORPHOLOGICAL FEEDING ADAPTATIONS AND
EXTREME SEXUAL DIMORPHISM IN ENERGY ALLOCATION IN INVASIVE LIONFISH
(*PTEROIS VOLITANS*)

ROBIN A. COSTELLO, NINA B. FRANKEL, AND MADILYN M. GAMBLE

Faculty editor: Celia Y. Chen

Abstract: Lionfish (*Pterois volitans*) are a rapidly spreading invasive marine species in the Western Atlantic. High feeding success and reproductive output are potential mechanisms that allow lionfish to be such successful invaders. In order to better understand these mechanisms, we studied the allometry of morphological features involved in lionfish feeding ecology (specifically pectoral fin length and gape area), using fat mass as a metric of feeding success. We expected shorter lionfish to have longer pectoral fins, larger gape areas, and more fat stored than expected for their length. This allometric pattern would give smaller lionfish a feeding advantage, allowing them to grow to a large size quickly and outcompete other reef piscivores. Since we expected increased pectoral fin length and gape area to increase feeding success, we expected lionfish with longer pectoral fins and larger gape areas relative to length to have relatively more fat. We found that (1) shorter fish have longer pectoral fins, larger gape areas, and more fat mass than expected for their size, (2) lionfish with longer pectoral fins and larger gape areas for their length do not have more fat than expected for their length, and (3) females of a given body size had significantly lower fat mass than males. Our results indicate that compensatory allometric scaling of morphological features involved in feeding may give shorter lionfish a competitive advantage over native piscivores. We also found that fat mass is not necessarily indicative of feeding success. Finally, adult females may be investing more energy in reproduction than in growth or fat storage. Because lionfish reproduce continually while other reef piscivores reproduce seasonally, this intense allocation of energy into reproduction may play a critical role in the invasive success of lionfish in the Caribbean.

Key Words: Lionfish, *Pterois volitans*, invasive species, reproductive success, allometry

INTRODUCTION

Biological invasions are homogenizing the world and occurring more frequently as human activity increases on an international scale (Carlton and Geller 1993). Despite the overall increase in non-native introductions, introductions of marine fish species are surprisingly rare (Cohen and Carlton 1998). Lionfish (*Pterois volitans*) are the first non-native marine fish species to invade the Western Atlantic (Morris and Atkins 2009, Whitfield et al. 2002, Fig. 1). This lionfish invasion represents one of the most rapid marine fish invasions in documented history (Morris et al. 2009). Anecdotal evidence blames the introduction of lionfish on releases from aquaria in South Florida (Whitfield et al.

2002). Since introduction in the 1990s, lionfish have spread north to Bermuda and south to Jamaica (Whitfield et al. 2002, Ruiz-Carus et al. 2005). Albins and Hixon (2008) performed the first experimental study investigating the impact of this invasion, demonstrating that lionfish predation decreased native coral reef fish recruitment by 79%. This study showed that lionfish feeding ecology is a vital factor in understanding the success of their invasion. Previous studies have documented that lionfish use their long pectoral fins to corral fish and benthic invertebrates into their mouths (Morris and Atkins 2009). Lionfish also blow a jet stream of bubbles to mimic a current, tricking fish into swimming headfirst into their mouths (Albins and Lyons 2012). Such novel predation strategies potentially



Fig. 1. *Pterois volitans* on the Owen's Island reef, Little Cayman Island. Photo by Nina Frankel.

allow lionfish to grow rapidly and outcompete other piscivorous fish for prey and resources, contributing to their success.

To investigate which characteristics of lionfish contribute to their feeding success in invaded communities, we measured two feeding-related morphological features: pectoral fin length and gape area. Additionally, we assumed that the mass of fat stores in a lionfish would serve as a metric of feeding success. If these morphological features and fat stores follow an allometric growth pattern where smaller fish have longer pectoral fins, larger gape areas, and/or larger fat stores than expected for their size, this would indicate that selection has favored greater prey-capture ability in smaller lionfish. Because this allometric pattern would give smaller lionfish a feeding advantage, lionfish could grow to a large size quickly outcompeting other reef piscivores. If lionfish's feeding success is directly related to feeding morphological features, we would expect lionfish with longer pectoral fins and larger gape areas for their size to have more fat stores than expected for their size. Alternatively, sex may be a better predictor of fat stores than morphological features. Unlike most piscivorous Caribbean fish, lionfish do not spawn once or twice a year (Rhodes and

Sadovy 2002). Instead, studies suggest that lionfish reproduce during all seasons (Morris et al. 2008) and may be capable of reproducing every four days (Morris 2009). Thus, if sex is a better predictor of fat than morphology, we would expect females to have less fat than males due to allocation of energy to reproduction instead of growth.

METHODS

On 1 March 2012, lionfish ($n=41$) were culled at the Rock House Bay dive site off Little Cayman Island. The fish were speared by divers at a depth of approximately 55 feet. On 2 March 2012, additional lionfish ($n=4$) were obtained at approximately the same depth from the Locher's dive site off Little Cayman.

On 2-3 March 2012, we dissected the lionfish, measuring mass, total length, gape height, gape width and pectoral fin length. Total length (TL) was measured as the distance from the front of the mouth to the end of the caudal fin. Gape length and width were measured from the inside edges of the mouth. Pectoral fin length (PFL) was measured from the base of the fin to its longest point. Fish mass was measured to the nearest 0.1 gram using an Ohaus Explorer balance. Gape area (GA) was obtained by multiplying gape height and gape width. We identified each lionfish as male or female based on observation of the gonads and removed the internal organs, including the intestines, liver and all fat (stomachs were removed separately). We cut out the lobes of fat from the rest of the organs and weighed the fat to a precision of 0.0001 grams using an Ohaus Adventurer balance.

Statistical Analyses

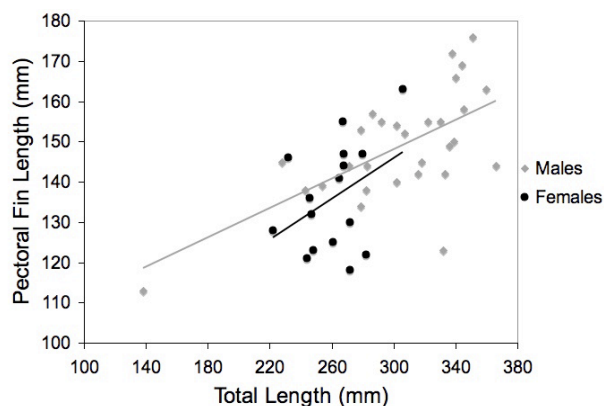


Fig. 2. Pectoral fin length significantly increases with total length for males but not for females.

Fat mass was square root transformed and GA was log transformed for normality. A multiple regression was used to determine which morphological characteristics (PFL, GA, TL, or sex) best predicted fat mass. To analyze various pairwise relationships, we regressed TL on PFL, GA, and fat mass, separately for male ($n=29$) and female ($n=16$) lionfish.

To account for the effect of fish length on PFL, GA, and fat mass, we took the residuals of each of the regressions mentioned above regardless of statistical significance. For the remainder of this paper, we will refer to these residuals as relative PFL, relative GA, and relative fat mass. We performed two linear regressions with relative PFL and relative GA as predictors of relative fat mass. These regressions were performed separately for males and females. We also calculated residuals of fat mass by length regardless of sex and performed a t-test to determine if this relative fat mass differed between males and females. All statistical analyses were performed using JMP 9 software (SAS Institute, Cary, NC).

RESULTS

The mean TL was 304 ± 8.8 mm for males and 261.3 ± 5.2 mm for females (mean \pm 1

S.E.). TL ranged from 138 to 366 mm for males and from 222 to 306 mm for females. Since lionfish reach sexual maturity at lengths of 100 mm for males and 180 mm for females (Morris and Whitfield 2009), all lionfish included in our study were adults.

The multiple regression analysis indicated that sex was the only significant predictor of fat mass ($t = -2.97$, $df=40$, $P=0.005$). None of the other main effects significantly predicted fat mass (TL: $t = 1.70$, $df=40$, $P=0.098$; GA: $t = -0.60$, $df=40$, $P=0.554$; PFL: $t = 1.58$, $df=40$, $P=0.123$).

For univariate analyses, PFL significantly increased with TL for males ($r^2=0.40$, $df=27$, $P<0.001$, Fig. 2) but not for females ($r^2=0.15$, $df=14$, $P=0.132$, Fig. 2). The slope of this regression for males ($m=0.18$) was less than 1. GA significantly increased with TL for both males and females ($r^2=0.93$, $df=27$, $P<0.001$; $r^2=0.62$, $df=14$, $P<0.001$, respectively; Fig. 3). The slope of these regressions for both males ($m=0.009$) and females ($m=0.008$) was less than 1. Fat mass significantly increased with TL for males ($r^2=0.41$, $df=27$, $P<0.001$, Fig. 4), but not for females ($r^2=0.01$, $df=14$, $P=0.7278$, Fig. 4). The slope of this regression for males ($m=0.01$) was less than 1.

Relative PFL did not significantly predict relative fat mass for either males ($r^2=0.06$, $df=27$, $P=0.209$) or females ($r^2=0.11$, $df=14$, $P=0.1995$). Relative GA did not significantly predict relative fat mass for either males ($r^2=0.03$, $df=27$, $P=0.367$) or females ($r^2=0.05$, $df=14$, $P=0.416$). Relative fat mass regardless of sex was significantly greater in males than females ($t=5.06$, $df=40.1$, $P<0.001$).

DISCUSSION

Our results indicate significant differences in growth allometries between male and female lionfish. However, since the slopes of all allometric relationships were less than 1 or

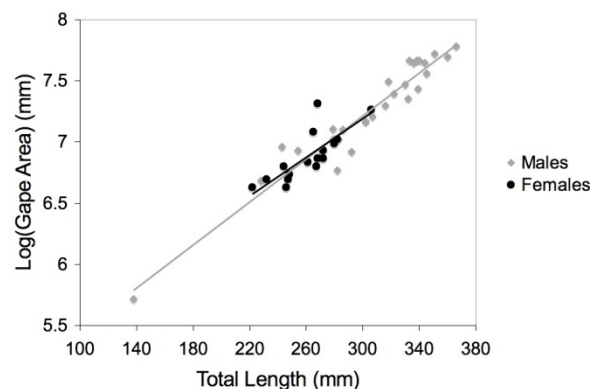


Fig. 3. Gape area significantly increases with total length in both males and females.

not significantly different from 0, smaller fish, regardless of sex, have longer pectoral fins, larger mouths, and more fat stores relative to their length. This suggests either that shorter lionfish may be compensating for their small size, or that shorter lionfish have been selected to have larger feeding features that could increase their feeding success. It is not known whether their piscivorous competitors (such as groupers, jacks, and snappers) exhibit similar allometric scaling of morphological features that are important for feeding. If lionfish are the only reef piscivores to display these compensatory patterns, shorter lionfish may be able to use their relatively longer pectoral fins and larger mouths to grow at a faster rate than their competitors.

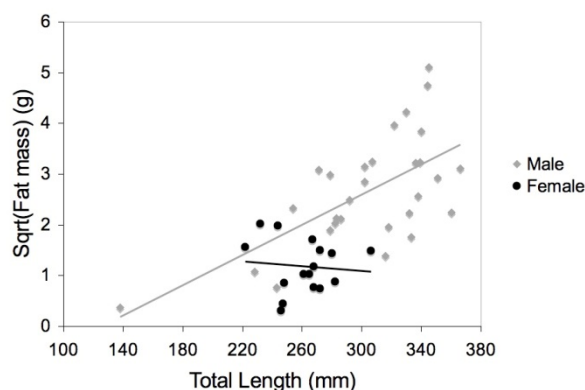


Fig. 4. Fat mass significantly increases with total length for males but not for females

Despite this potential compensatory mechanism, the fact that lionfish of both sexes with relatively longer pectoral fins and larger mouths do not have relatively more fat suggests that these morphological characteristics may be less important to feeding success than we had hypothesized. Feeding success could depend more on diet or behavioral adaptations than on morphological features. Since small lionfish primarily eat crustaceans and large lionfish are primarily piscivores (Morris and Atkins 2009), diet may affect fat stores in differently sized lionfish. Also, predation pressure may play a greater role than feeding success in selecting for relatively longer pectoral fins in shorter lionfish. Alternatively, prey may not be limiting lionfish in the Western Atlantic. A lack of interspecific competition for food could explain why lionfish with relatively longer pectoral fins and larger mouths do not have more fat than their less well-endowed conspecifics.

Fat stores may not be a good indicator of feeding success in lionfish and could be influenced more by energy allocation than by feeding success. The fact that TL does not predict PFL or fat mass in females suggests that female lionfish are allocating energy differently than males. Females may switch from investing energy in somatic growth and fat storage to investing in reproduction once they reach sexual maturity, as occurs seasonally in other fish species that spawn once or twice a year (Reznick and Braun 1987). Lionfish reproduction is not well studied, but it is estimated that lionfish can produce between 20,000 and 30,000 eggs as often as every 4 days, year-round (Morris 2009). This would require a relatively constant energy allocation to egg production in females, which would explain why females have significantly lower fat stores than males.

Given the fitness trade-off between investing in reproduction and survival, it is surprising that female lionfish do not face extremely high mortality in exchange for allocating so much energy towards reproduction. Release from predation, disease, and parasites in the Western Atlantic post-introduction (Ruiz-Carus et al. 2006) could allow female lionfish to invest high amounts of energy into reproduction without incurring survival costs.

Feeding ecology and reproductive success are vital components in understanding how non-native species invade certain ecosystems. Compensatory allometry of morphological features in smaller size classes could give lionfish a competitive advantage over other coral reef predators, contributing to their success as invaders. The apparent switch in energy allocation from growth and fat stores to reproduction in adult females warrants future studies including both juveniles and adults. In female lionfish, the apparent intense energy allocation into reproduction without decreased survival likely contributes to the incredible success of lionfish as an invasive species in the Caribbean. Future studies should investigate the trade-off between reproduction and survival in lionfish in their native environment to see how it compares to Western Atlantic populations. The combination of abnormally high reproductive success with morphological adaptations that could give smaller lionfish a feeding advantage over other reef piscivores may play a crucial role in the invasive success of lionfish in the Caribbean.

ACKNOWLEDGEMENTS

We would like to thank Savanna Barry for her guidance and expertise on invasive lionfish, and for including us in her weekly lionfish dissections. We would also like to

thank Katie Lohr for her help with dissection of lionfish, and the CCMI staff and volunteers for their hospitality and support.

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SIZE AS AN INSUFFICIENT PREDICTOR OF DUSKY DAMSELFISH (*STEGASTES FUSCUS*) TERRITORIAL AGGRESSION

SHEA E. FLANAGAN, MADELINE K. KREHER, AND JAMIE E. MCCLAUGHLIN

Faculty editor: Celia Y. Chen

Abstract: Game theory posits that the decision to engage in an aggressive interaction should depend on the perceived probability of success and therefore, competitive interactions tend to favor the larger competitor. We conducted a study to determine whether larger individuals of the territorial Dusky Damselfish (*Stegastes fuscus*) will react sooner and attack more frequently than smaller conspecifics, and that aggression will increase as the size of the intruder decreases. We simulated intruders 40% and 75% smaller than the host damselfish by placing either one of two convex mirrors, or a shiny, but not reflective control, in 21 damselfish territories in Grape Tree Bay, Little Cayman. We found that individuals reacted to both mirror treatments but not to the control. Neither fish size class nor mirror treatment invoked a more rapid reaction time or greater attack rate. Overall, dusky damselfish risk their safety to defend their territory and resources, which are highly valued in the competitive coral reef ecosystem, regardless of intruder size.

Key Words: aggression, game theory, home field advantage, *Stegastes fuscus*, territoriality

INTRODUCTION

Competitive interactions between organisms in nature are widespread. Organisms most often rely on aggression, defined as a hostile behavior towards another organism, to defend resources such as feeding territories, but this behavior carries costs. Aggressive resource defense increases the risk of injury and predation, detracts from mating and foraging time, and drains energy reserves (Cleveland 1999). An aggressive strategy is advantageous only if the benefits outweigh the costs, but benefits differ dramatically contingent on the outcome. Game theory predicts that the decision to engage in an aggressive interaction should consider the potential outcome of the conflict (Maynard Smith and Price 1973).

Size differences between competitors often mediate the outcome of competitive interactions. Given similar fighting tactics, larger individuals triumph more frequently than smaller individuals (Hughes 1995). With greater chance of success, a larger individual risks fewer costs when engaging in aggressive

behavior. Therefore, we predict that larger individuals will engage in aggression more quickly and more pugnaciously. Furthermore, we expect that aggression will increase as the size of the competitor decreases.

Dusky damselfish are an appropriate study system to investigate our predictions because both competitors possess similar fighting tactics and abilities relative to their size. Dusky damselfish vigorously protect their algae grazing grounds from conspecific intruders and other herbivores by charging those that approach their 0.37 to 1.86 m² territories (Deloach and Humann 1999). Because of their determined defense of algal turfs, dusky damselfish play an important role as ecosystem engineers—both increase algal biomass and benthic invertebrate diversity (Mahoney 1981, Ferreira et al. 1998).

METHODS

We investigated intraspecific aggression in the territorial, reef-dwelling dusky damselfish (*Stegastes fuscus*). On 2 - 4 March 2012, we identified 21 dusky damselfish in the back

reef of Grape Tree Bay on Little Cayman Island. We haphazardly selected 7 adult fish each of small (less than 7.5 cm), medium, (between 7.5 and 10 cm) and large (greater

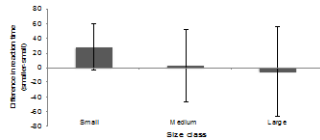


Figure 1. *Stegastes fuscus* size did not predict individual reaction time to perceived intruder. Individuals did not react to either small or smaller mirror faster nor did they react differently between host size class. Error bars represent means \pm 1 SE.

than 10 cm) size classes. To simulate an intruder, we placed two convex mirrors in each fish's territory. Each fish received three treatments in a randomized order: a control and two mirrors that simulated intruders of different sizes. The control was a piece of aluminum foil that functioned as a shiny, but not reflective, object of similar size to the mirrors. The "small" mirror presents a perceived intruder 60 % the size of the host, while the "smaller" mirror presents a perceived intruder 25% the size of the host when the mirrors were placed 0.3 m from the observer. We recorded how long it took for the fish to react to its reflected image (reaction time) and counted the number of attacks per minute (attack rate) for the first two minutes after the initial attack. We defined an attack as the fish making contact with the mirror. If the fish did not attack after five minutes, we stopped observation and recorded reaction time as 300 s, the total length of the observation period. We waited 5 minutes between the treatments of each fish to allow the fish to return to its normal behavioral state.

Data Analysis

We calculated the difference between each fish's reaction time on the 60%-sized intruder and the 25%-sized intruder to explore the

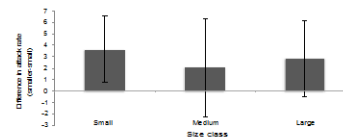


Figure 2. *Stegastes fuscus* size did not predict the attack rate of individuals against a perceived intruder. As individuals did not react to either small or smaller mirror more nor did they react differently between host size class. Error bars represent means \pm 1 SE.

effect of perceived size of intruder. We then conducted a oneway ANOVA between this difference in reaction time and fish size class. Finally, to measure whether the perceived size of the intruder determined the attack rate of the host fish, we calculated the difference in attack rate on the 60%-sized intruder and the 25%-sized intruder for each individual. We then conducted a oneway ANOVA between this difference in attack rate and size class.

All analyses were performed using JMP 9.0 (SAS Institute, Cary, NC).

RESULTS

We observed dusky damselfish fend off intruders much larger than themselves, such as parrotfish and sergeant majors, as well as other dusky damselfish. We also observed that only one individual, in 21 trials, reacted to the control by attacking it once, thus confirming that damselfish only attack a perceived intruder rather than any object placed in their territory.

Reaction time did not differ between treatments, nor did individuals of one size

class respond faster relative to the other size classes (ANOVA, $F_{2,19} = 0.13$, $p = 0.88$; Fig. 1). Attack rate did not differ between treatments, nor did individuals of one size class attack more frequently than the other size classes (ANOVA, $F_{2,19} = 0.05$, $p = 0.95$; Fig. 2). However, we found that small fish attacked the 60%-sized intruder less frequently than medium and large fish (mean small attack = 6.57 attacks/min, mean medium and large attacks = 12.23 attacks/min), but this relationship was not significant ($p = 0.24$).

DISCUSSION

Our results suggest that damselfish territorial behavior does not change with either competitor or host size. If size is a factor in determining outcomes of competitive interactions over the scale we measured, then this observation counters the predictions of game theory. Our results suggest that the tradeoffs associated with territorial responses do not prove costly enough to deter damselfish from engaging in fight. One possible explanation is that territory is particularly valuable in the crowded coral reefs and so the benefits of protecting these scarce resources overcome the costs.

Alternatively, size may not be an effective predictor of conflict outcome and thus is insufficient to explain game theory dynamics as it seems other factors favor the host regardless of intruder size. This result would support the importance of home field advantage or priority effects, which emphasize the importance of occupying a territory first as a means of successfully maintaining it. For example, Bredell (2009) found that in territorial rock bass, the intruder always flees the agonistic host. This explanation is corroborated by our

observations that dusky damselfish successfully excluded much larger intruders than themselves from territories, such as sergeant majors and even terminal phase parrotfish. Future studies could consider this observed behavior and conduct similar experiments using methods that manipulate the perceived intruder size on a greater scale to explore the possibility that damselfish will attack regardless of intruder size. Similarly, future studies should consider sampling a larger number of damselfish to increase the likelihood of detecting significant treatment effects.

It is interesting that dusky damselfish have persisted through evolutionary time by practicing aggressive territorial responses to a range of intruder sizes. Home field advantage, an evolutionary stable strategy that favors the established resident organism over an intruder, may explain the success of the host damselfish against intruding conspecifics in the context of game theory. However, it does not explain the success of a 7 cm damselfish overcoming a parrotfish over three times its side. One would assume that such high aggression could prove detrimental in the long term because damselfish likely expose themselves to predators when fending off larger fish. Perhaps damselfish rely on cues other than size, such as shape of the intruder, to assess the risk and outcome of the conflict and thus avoid aggressively attacking predators. In conclusion, the evolutionary persistence of the tiny damselfish's territorial behavior appears counterintuitive, yet emphasizes the complexity of predicting the outcome of competitive interactions.

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TRACK CHANGES: THIS TITLE DOES NOT ACCURATELY REFLECT YOUR HYPOTHESIS

MILO JOHNSON AND BENJI KESSLER

Faculty Editor: Milobus Johnson and Benjamin Kessler

Abstract: Remember, you still need to write an abstract.

Key words: wrong font, should be italic, capitalize, no comma here

INTRODUCTION

This first sentence isn't broad enough – widen the funnel here. You mention *Phacius speciesii* in the second sentence, which is far too early. You shouldn't even mention your study system until the second paragraph. OK, now you've introduced the Optimum Range Dispersal Theorem and the Macroevolutionary Species Dillusion model, but you need to expand on these theoretical frameworks. You cannot expect that the reader already understands Variable Mutualistic Selection theory. Now you need to link these ideas together and somehow make it seem like your study actually relates to these systems.

Really? How do you know that these animals actually eat the fruit? You need a citation here. This bit about the polyandrogenerotic mating system is interesting, but really has no place in this paper. Delete. This background about global warming is nice, but you're citing a source from 1893 in a journal that was neither peer-reviewed nor published. Why did you choose this study system again? And I don't see how aggressiveness in response to human attack relates to sexual selection.

You should really combine these two paragraphs. State your hypothesis clearly here but don't use the word hypothesis. This thinking is fairly good I

suppose but it seems like you could have managed to write this sentence with a couple fewer words or use other techniques to cut it down in the future. This sentence weren't in the write tense. Tighten it up guys.

METHODS

When? Where? Using what tools? Did you use your hands to do that? Don't be so specific here.

Data Analysis

You used a Gümthür-Hümtenfrüeight multiple inverse variable variables analyses? I think in this case (nonlogarithmic poisson antinormal zero skewed outliers) you should've been using a more appropriate test, maybe a Carviel-Ruxley Point Test Confusion Matrix or a Pee Test. Please don't use the

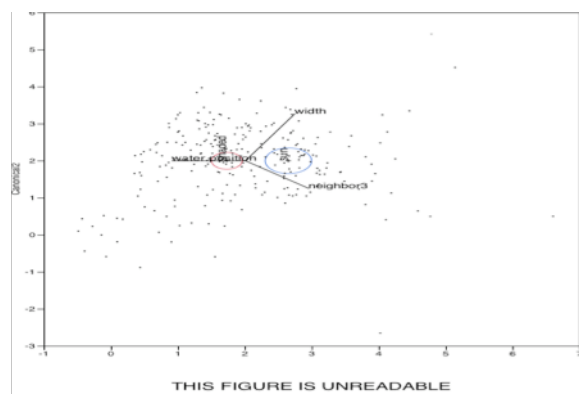


Fig 1. Just state the two variables separated by "and." Also, relate the variables and say something meaningful based on your results, but also don't do that and just report them. Just make sure to include the equation, r-squared, slope, p-values, etc. Statistics don't belong in figure captions.

statistician's methods without first reading and studying the 800-page book about them.

RESULTS

This result is suspect ($P < 0.0001$). You cannot report both a multiple correlation and a bilinear regression on non-non-parametric data (${}^3\text{CE}_{911} = -0.72$, $\text{SS}_{-2.8} = 0.47$, Fig. 1).

Your Helmholtz Q-value should be negative... what's going on here? You may have entered your data incorrectly. This kind of comment about the ultimately negative economical and social effects of your findings belongs in the discussion.

DISCUSSION

This first sentence of the discussion talks about a correlation between pregnancy and storks, but I didn't see anything about that in your intro, methods, or results. The conclusion that your data supports evolution is just not true. Delete.

Expand on your results here, I think you can discuss more about the lack of difference. I like your logic, but you really can't say from your study that the two species are competing, coexisting, co-occurring, or extant. You should suggest that a future study repeats your experiment with better methods, but be a little vague about it so you don't sound like a terrible scientist who just passes off work to the next year of students.

Don't end on this note. Widen the funnel. Widen the funnel. Widen the funnel. Great sentence here to wrap it up guys; I think this paper is really, really good, you just need to make a few quick changes and reframe your whole logical flow to focus on a completely

different theoretical context.

LITERATURE CITED

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COUPLES ARE BIGGER IN THE TROPICS: TESTING FUN SELECTION THEORY IN
EFESPIUS LAYNEII

MADILYN M. GAMBLE, BENJAMIN J. KESSLER, MADELINE K. KREHER, AND AMY E. VAN
SCOYOC

ABSTRACT: Read the damn paper.

Key words: conservation biology, *E. layneii*, ethanol, fun, fun selection, green earth hypothesis

INTRODUCTION

In the history of life on earth, even before the Cambrian explosion and the mass radiation of species, fun has been an essential component to ecosystems. The tropical nerd, *Efespius layneii*, is known to have fun parties on occasion (Monteverde 2012; Las Cruces 2012), but occasionally goes to bed too damn early (Gamble, 2010, 2011, 2012). Though the definition of sexual selection has been subject to little contention, we believe that its roots lay within a much more fundamental theory: "fun selection." Fun selection is manifested in secondary characteristics such as ornamentation and costumes. We believe these features to be the ultimate drivers of fun in the tropics. Furthermore, variable lek composition (2:1 or 1:2 male to female ratio) has been shown to induce disruptive selection (CITE). Based on the theory that couples are bigger in the tropics, we propose an experiment whereby we will test the fun success of variable lek composition in *E. layneii*.

METHODS

On 14 Feb. 2012, at precisely 19:30 we gathered 5 leks of various composition from the La Selva Biological Station (Table 1). Leks were allowed to sit for 6 hours until, upon their own volition, they chose to arrive in costume. Those that did not arrive in costume were spat on and excluded from the study as outliers. Upon arrival, Matt Ayers, an expert in the field, judged the success of each group by overall costume ornateness. Fun ensued.

RESULTS

Better costumes led to more fun ($r^2 = 1.0$; $p = 0.0$).

DISCUSSION

Discussion was held but not recorded.

LITERATURE CITED

Who cares, You. 2012. Fun. Nature. 1: 267-345.

TABLES AND FIGURES

Group	Person 1	Person 2	Person 3
1	Benji Kessler	Robin Costello	Amy Van Scoyoc
2	Madeline Kreher	Mike Logan	Madilyn Gamble
3	Sam Fey	Nina Frankel	Milo Johnson
4	Hem Chowdaury	Jamie McLaughlin	Wales Carter, Naturalist
5	Jesse T. "Rex" Rieb	Shea Flanagan	Anna "Orca Pirate" Deffebach

MARINE ECOLOGY IS JUST HARDER THAN TERRESTRIAL ECOLOGY

MILO S. JOHNSON

Faculty Editor: Benji Kessler

Abstract: People often lament the fact that while we know so much about terrestrial ecosystems, we have very little knowledge of marine ecosystems. Many hypotheses have been proposed to explain this phenomenon, but in this paper I propose that it is mainly due to the fact that marine ecology is just way too hard. First, I examine some of the immediate problems encountered in marine biology, such as the water-breathing conundrum and the wind-cold-experience hypothesis. Next, in a metaexperiment, I examine the differences between terrestrial and marine experimental design, showing that the design of a terrestrial study is much more likely to be sound and reasonable than that of a marine study. Finally, I urge all scientists with low scientific self-esteem to pursue terrestrial projects based on an analysis of the psychological effects of attempting marine studies.

Key words: waves, water, current, problems, cold, hard, not worth it

INTRODUCTION

For millennia, man has studied the world around him: the apples growing above, the ants scurrying below, and the birds flying far off on the horizon. Ever since Darwin, we have understood the biological world in the context of evolution, and pried at small and large scales to understand the mechanisms underlying life. Most of this research has been concentrated on landmasses, where observations are made simply and data is easily obtained. Recently however, there has been a trend in some circles to begin studying the ocean. Startlingly, papers have been published, and “results” have been reported (Mumby 2001, 2002, 2004, 2005, 2006a, 2006b, 2008, 2010, 2011).

Even simple, one-day marine studies on subjects like sea grasses have sparked immediate negative reactions in young researchers (Costello 2012). Long-term

studies experience similar problems, from wave-action tumbling to uncontrollability issues. Several researchers have also noted that a significant complication for marine research involves the apparent inability of humans to breath in the ocean (Johnson and Kessler 2012).

In this study I investigate the relative success and implications of marine research in comparison to the classic terrestrial system.

METHODS

This is a review; I did no research or data collection and just wrote this.

RESULTS

Studies conducted in marine environments had less controlled variables and smaller sample sizes than comparable terrestrial projects (fig. 1, $p=0.0001$, $p=0.021$).

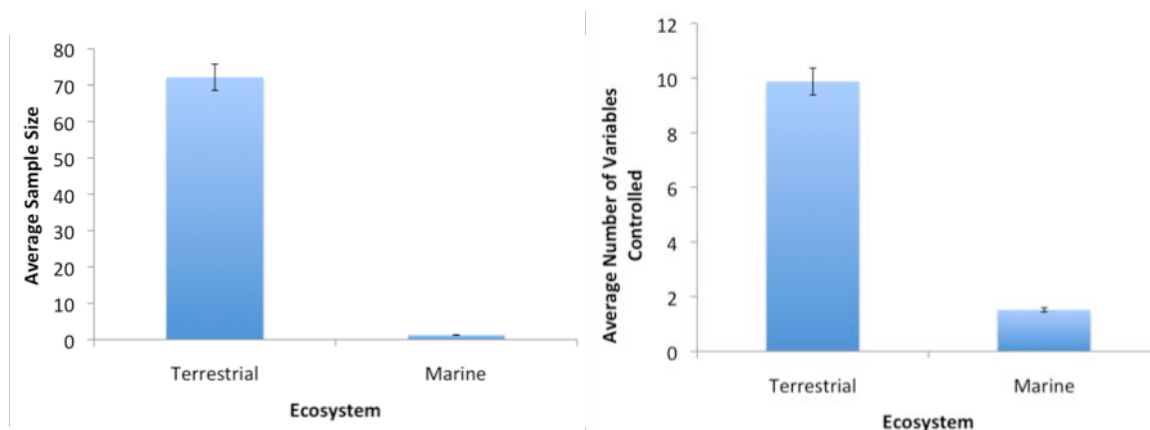


Fig 1. Average sample size and average number of variables controlled were way, way lower in marine ecosystems.

Breathing ($p < 0.0001$), moving ($p = 0.003$), recording data ($p = 0.004$), observing the study system ($p = 0.005$), and avoiding injury ($p = 0.04$) were all more difficult in marine projects. There was a trend that saltiness was

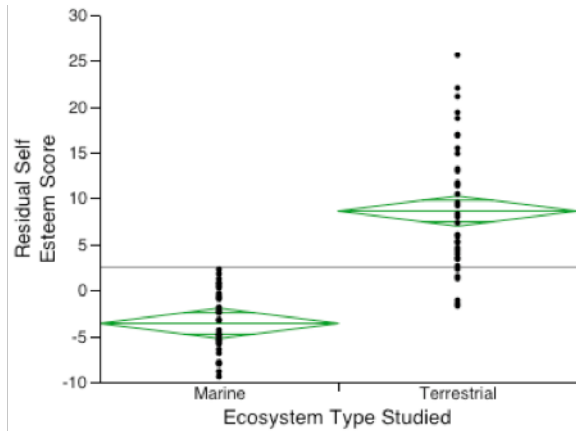


Fig 2. Researchers had significantly lower residual self-esteem scores after attempting marine projects. Data collected using facial expression analyses and emotional conversations.

higher in marine projects ($p = 0.050001$), but it was not significant. I have plotted (fig. 2) the success of one foreign study program as students worked on terrestrial projects for 7 projects and then completed a marine project. The corresponding residual self esteem scores were significantly lower both during and after the marine project (fig. 3, $p = 0.0003$). Marine methods were also significantly different from terrestrial systems. To create a properly controlled environment in a marine system, a researcher had to provide 30 glass aquaria with pumped running ocean water of an exact PH, temperature, salinity, and

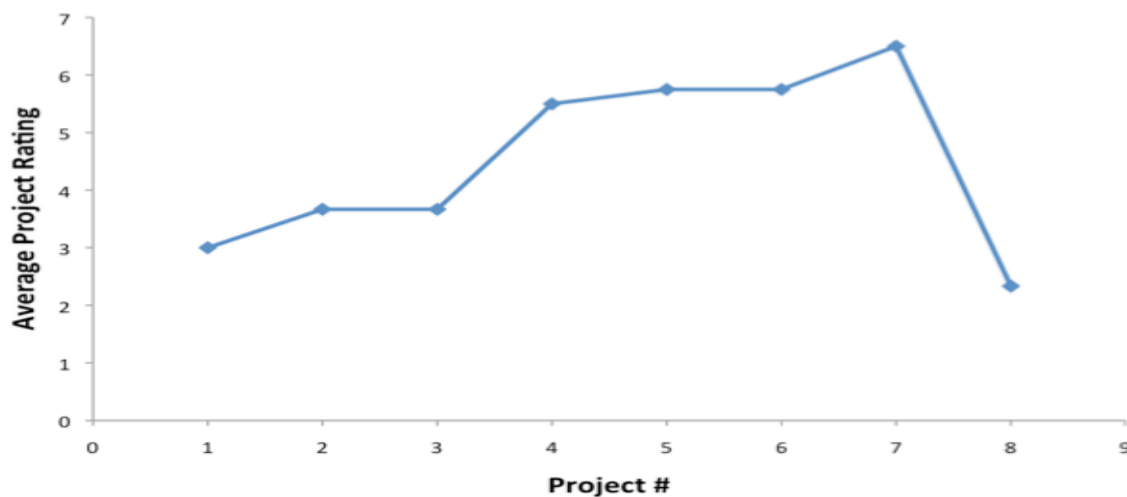


Fig 4. Average ratings of student projects on a Dartmouth foreign study program. The first seven projects were completed in terrestrial ecosystems, while the eighth was completed in a marine ecosystem. Researchers with especially low scores (Johnson and Kessler 2012) were asked to leave the island.

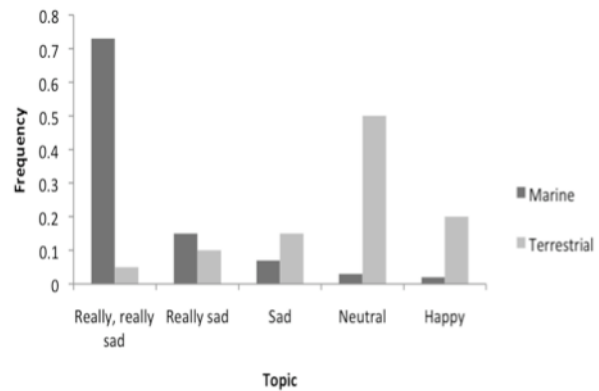


Fig 3. Frequency distribution for the relative outlook of marine and terrestrial projects. Marine projects were usually really, really sad, while terrestrial projects were mostly neutral. Data was collected using a volumetric flask to catch tears.

dissolved oxygen level, and control for day-night cycles as well. In a terrestrial system, a researcher had to look at flowers while drinking beer.

DISCUSSION

Marine ecology has been generally viewed as the dark, angry teenage step-brother of terrestrial ecology, pessimistic and difficult. My results suggest that marine studies are, in fact, more difficult and less successful than similar terrestrial studies. In addition, marine papers were generally wholly depressing when compared to terrestrial papers (Mumby 2003, all other FSP critiques), and the self-esteem of researchers

usually dropped precipitously when researchers attempted marine studies. Further research is needed to determine whether this stems mainly from the decline of beautiful coral reefs and other marine species or simply the sadness acquired from spending 16 years swimming transects for one project.

The root cause of these differences is difficult if not impossible to pinpoint, but I suspect that the answer lies in the fact that marine projects take place in the ocean, and humans do not live in the ocean. The ocean itself is a harsh environment, without simple amenities like air, stability, or potable water. Though being in the ocean has long been considered “fun” by beachgoers, researchers have found that when going into the ocean is necessary, even in bad weather, being in the ocean can become considerably less pleasant and data collection can become difficult (Costello *et al.* 2012, Chowdhury *et al.* 2012).

The creatures of the ocean hold many mysteries behind their colorful patterns and dull, stupid eyes. Will we ever know what that species of fish actually eats? Which invertebrate clade will go extinct next? If I break off an entire section of reef, move it somewhere else, and introduce a terrible

invasive species, will I feel bad about myself at all? For now, the mysteries remain mysteries. To solve them we will need a new generation of scientists who are willing to struggle for years to collect any reasonable data, make countless assumptions, and swim miles. Will we find them, or will we leave the sea as a mysterious, unscientific frontier? It certainly seems easier just to stay on dry land.

LITERATURE CITED

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TITLES OF REJECTED OR UNFINISHED PAPERS
Compiled by Robin Costello

FOREWORD: The following pages contain the working titles of several studies that were either too good or too failed to appear published in this volume. Though unpublished, each of these studies represents dozens of man-hours of grueling labor. As such, their titles are presented here to commemorate the otherwise unrecognized effort put into these projects.

- ❖ Acacia were wondering: We didn't find out anything new about acacia ants
- ❖ Close encounters of the brittle kind
- ❖ Lung usage strongly linked to success in bug-catching
- ❖ Tapir by the dozen
- ❖ Coati of arms: allometric scaling in procyonids
- ❖ The effects of biotic and abiotic factors on the activity of organisms: a study of the diel activity patterns of *Pseudomyrmex spinicola*
- ❖ Local global warming on *Siderastrea siderea*
- ❖ Black vultures: a study on black vultures
- ❖ Gamma diversity between beach and swimming-hole habitats in Corcovado national park
- ❖ El cangrejo rojo: Se llama "cangrejo rojo" porque es rojo
- ❖ Two snakes enter, one snake leaves: interspecific competition in a lab setting of two pit viper species
- ❖ Five snakes enter, one brittle star leaves: Surprising phylogenetic relationship between Ophiuroids and Serpentes
- ❖ Spray it again, Sam: effectiveness of different brands of mosquito repellent
- ❖ Whale sharks: Behavior, feeding, and absence from our study *Rhincodon typus*

- ❖ *Rhincodon typus*: Biggest fish in the ocean, smallest sample size on the FSP
- ❖ A study in the untamed tropical wilderness: Economics and fiscal feasibility of farming the North American lake trout
- ❖ A Neotropical Passion: Love and betrayal in the new world tropics
- ❖ I hope God is not a beetle: Effects of flowers on the internal thoracic temperatures of several hapless chrysomelids
- ❖ Dr. Chitonlove, or how I stopped worrying and learned to love invertebrates
- ❖ On the increase of pectoral fin size with decrease in length in male lionfish and on their feeding behavior and how this relates to their fat stores and morphology and sexual dimorphism and costs of reproduction and several other factors related to and describing the behavior and morphology of lionfish: A study on an invasive species
- ❖ The fastest and smartest lizard in the world: How *Ctenosaura similis* outperformed us physically and mentally, then made two of our leaders bleed
- ❖ *Dysdercus circus*: It turns out bugs can't see the color red
- ❖ Poisson wood bible: There is nothing interesting about the habitat selection of termites
- ❖ Extinction in the tropical montane cloud forest: When exhaustive sampling goes tragically wrong
- ❖ Humming all over each other: Territoriality in hummingbirds
- ❖ Lizard switching as a method to not catch lizards: Never switch lizards
- ❖ Great Danes: Shoe-watching behavior of a migratory myridologist
- ❖ Booster shots: Professorial habits of an inferior rapper